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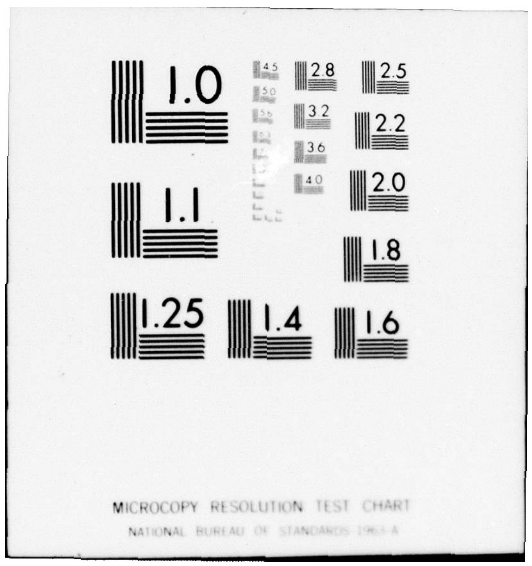
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REPORT NUMBER 61-0614-77

DEVELOPMENT OF GROUND AND AERIAL ADULT CONTROL MEASURES FOR BITING DIPTERA WITHOUT USING PERSISTENT PESTICIDES

FINAL REPORT

BERNARD A. SCHIEFER, RALPH R. CARESTIA, ROBERT W. VAVRA, JR.
AND ROBERT L. FROMMER
Radiation and Entomological Sciences Branch
Regional Division - North
US Army Environmental Hygiene Agency
Fort George G. Meade, MD 20755

APRIL 1977

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US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Field investigative studies, spanning a 3-year period, were conducted to minimize the nuisance caused by biting black flies at Fort Drum, NY. Ground and aerial chemical control measures were employed with little success. The prevention of bites through the use of repellent impregnated jackets was most promising. Additionally, survey techniques and species bionomics were studied and some laboratory studies were conducted to assist in control measures. Several preprints and reprints of results from these studies are included.

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DEPARTMENT OF THE ARMY
U.S. ARMY ENVIRONMENTAL HYGIENE AGENCY
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FORT GEORGE G. MEADE, MARYLAND 20755

HSE-MM

REPORT NO. 61-0614-77
DEVELOPMENT OF GROUND AND AERIAL ADULT CONTROL MEASURES FOR BITING DIPTERA WITHOUT USING PERSISTENT PESTICIDES
MARCH 1973 - JUNE 1976

ABSTRACT

Field investigative studies, spanning a 3 year period, were conducted to minimize the nuisance caused by biting black flies at Fort Drum, New York. Chemical control utilizing aerial spray was attempted over both small and large areas using six different pesticide formulations. Control was not successful because of the difficulty in timing spray flights when black flies were most active and the ability of black flies to rapidly re-enter sprayed areas. Chemical control by ground application of six different pesticide formulations was equally unsatisfactory primarily due to poor penetration into the target area. Parameters influencing black fly flight activity were defined and used in conducting various phases of the study. Survey techniques evaluated included sweep count, landing rate count, estimation rate, and modified light traps using carbon dioxide as an attractant. Of these, modified light traps using a flow rate of 500 milliliters carbon dioxide per minute offered the most effective survey tool for monitoring adult black fly populations. As a means of providing personal protection, mesh jackets impregnated with deet and twelve experimental repellents were extensively tested. Because of current restrictions on the selection of pesticides and methods which can be used against black flies and the unsatisfactory results of pesticide application techniques reported herein, personal protection afforded by repellent jackets appears most promising. Recommendations include the introduction of an insect repellent jacket into the defense supply system. Six articles related to this subject have been published under this contract and one additional publication is expected.

Rept No. 61-0614-77, Dev of Ground and Aerial Adult Control Measures for Biting Diptera Without Using Persistent Pesticides, March 1973 - June 1976

CONTENTS

DEPARTMENT OF THE ARMY
U.S. ARMY ENVIRONMENTAL HYGIENE AGENCY
REGIONAL DIVISION - NORTH
FORT GEORGE G. MEADE, MARYLAND 20755

HSE-MM

REPORT NO. 61-0614-77
DEVELOPMENT OF GROUND AND AERIAL ADULT CONTROL MEASURES
FOR BITING DIPTERA WITHOUT USING PERSISTENT PESTICIDES
MARCH 1973 - JUNE 1976

1. REFERENCES.

a. AR 40-5, Health and Environment, 25 September 1974.

b. Letter, AHDA, First United States Army Medical Laboratory, 4 December 1972, subject: Request for Medical Research Support.

2. PURPOSE. To develop control and/or preventive measures to minimize the nuisance caused by biting black flies at Fort Drum, New York.

3. GENERAL.

a. Background. Black flies present a severe nuisance to military personnel engaged in training activities at several military installations located within this Division's geographical area of responsibility. Specifically, black flies have seriously interfered with annual field training activities of some 90,000 Army Reserve and National Guard units which train at Ft Drum, NY, during the spring and early summer months each year. The persistent problem affects not only the morale of the soldier but also reduces the effectiveness of the unit training exercises. In addition to providing information to alleviate this problem in training areas, any information developed concerning black flies would have military significance in other areas of the world. Certain species of Simuliidae transmit onchocerciasis in Africa and several Central American countries, some species cause severe allergic reactions in man in South America, and many others present a severe nuisance to man in portions of the temperate region.

b. Support. Funds to support this field investigative project were requested from the US Army Medical Research and Development Command (USAMRDC) on 4 December 1972. The project was approved and funded on 14 March 1973 and continued until 30 June 1976. The amounts provided by the USAMRDC were $10,163 in 1973, $10,388 in 1974 and $7,370 in 1975 for a total of $27,921 over the 3 year period.

4. FINDINGS AND DISCUSSION. The overall approach taken to investigate the problem is shown in Figure 1. During the first year of the study, ground and aerial chemical control measures, repellent jackets and various sampling methods were evaluated. During the second year, studies were focused on the effectiveness of aerial spray over a larger area, on evaluation of modified light traps as a survey tool and on the effectiveness of repellent jackets under field conditions. During the last year of the study attention was given to evaluating various repellents for personal protection, to laboratory studies in support of biological control possibilities and to refinement of a modified light trap survey technique. Description and results of the various aspects of the study follow.

a. Aerial Dispersal.

(1) 1973.

(a) To determine the effectiveness of black fly control by aerial spraying of various nonpersistent insecticides over comparatively small areas (5 mi^2 plots), four spray missions were flown by the United States Air Forces Special Aerial Spray Flight Unit, Lockbourne Air Force Base, Ohio, during the period 24 May - 14 June 1973. The insecticides tested were malathion and naled. Butoxy polypropylane glycol, a fly repellent, was added to naled for one treatment. Malathion was applied at two different rates. Four test plots and one control site, each 5 mi^2 in area, were selected for the evaluation.

(b) The results of these studies are described in detail in Appendix A. The use of aerial spray for black fly control in the 5 mi^2 plots was judged unsatisfactory. Poor control was attributed to many factors including poor meterological conditions for application and the rapid reinfiltration of flies from untreated areas. The investigators found that temperature, wind speed, and light intensity had the greater effects on control efforts because of their direct correlation with black fly activity. They concluded in addition to spraying at ideal weather conditions, larger areas should be sprayed to prevent rapid re-entry and infiltration into the target area and that other formulations should be tested with the aerial spray technique.

(2) 1974.

(a) A technical objective during 1974 was to continue to evaluate the efficiency of aerial spraying as a means of area control for black flies using various solvents such as heavy aromatic naptha (HAN) and other additives with naled. On 4 June 1974, 10,240 acres were treated with naled at a rate of 1 oz per acre. On 21 June 1974, 17,280 acres were treated using 9 oz of HAN plus 1 oz of naled per acre.

(b) Results of these studies are shown in Appendix B. Caged specimens were killed within 20 minutes after being sprayed with naled plus HAN, but not with naled alone. The investigators felt that 7-10 days control could be achieved using this formulation; however, the adult population was on a natural decline at the time of the second spray and it was not possible to accurately determine the effectiveness of the aerial spray.

b. Ground Dispersal.

(1) Ground dispersal of insecticides was evaluated only during 1973. Seven test sites to include one control area was used; each was approximately 1 mile long and 300 ft wide. The following insecticide formulations were utilized: 33.8 percent malathion plus 14.5 percent Lethane®; Pyrocide 7067® (5 percent pyrethrins plus 25 percent piperonyl butoxide); Pyrocide X2749 (5 percent pyrethrins, 25 percent piperonyl butoxide plus 5 percent repellent II); 85 percent naled; 9.3 percent naled; and 6.5 percent propoxur. An experimental rotary tube sprayer providing an ultra low volume (ULV) aerosol was used for dispensing the chemical compounds.

(2) Results of the ground ULV spraying are described in Appendix C. On caged black flies, naled was the most effective compound tested producing 100 percent mortality within 3 min at a distance of 225 ft downwind. At that distance all other compounds except malathion plus Lethane caused some mortality. However, the natural populations at all sites were not reduced for periods longer than approximately 5 minutes. The investigators felt that the sprayer did not provide sufficient penetration of the aerosol particles into the vegetation. Also, the small areas treated allowed for rapid re-entry of the black flies from untreated areas.

(3) In a separate test, the effectiveness of the insecticide resmethrin was evaluated against caged black flies. Complete mortality was obtained in approximately 5 minutes. These data are presented in Appendix D.

c. Survey Techniques.

(1) 1973.

(a) One of the original technical objectives was to develop and apply sampling methods to assess the magnitude of the problem and later the effectiveness of control measures. An informal evaluation was made of the sweep count, the landing rate count, and the estimation rate. Landing rates were determined by counting the number of flies landing on the upper

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torso in a given period of time. The sweep counts were made in two ways: continuously for a period of time (1 min), and by making a given number of sweeps about the head and body, generally 10 or some multiple of 10. The estimation method was evaluated by estimating the number of flies around the head after standing still or walking around slowly for a certain length of time. The investigators felt that none of these methods were satisfactory. Considerable bias in the population estimates by different observers was unavoidable. Plans were made to try modified mosquito light traps for the next year's study.

(b) Observations indicated that the more important parameters affecting adult flight activity were temperature, light intensity and rainfall. The effect of these are described in Appendix E. Black fly species occuring in the Ft Drum area include *Prosimulium hirtipes* complex especially *P. mixtum*, *Simulium vensustum*, and *Cnephia mutata*. A brief description of these species can be found in Appendix E.

(2) 1974.

(a) Studies were continued to investigate various adult survey techniques which were needed to better evaluate the effectiveness of aerial and/or ground applications of nonpersistent insecticides. Two mosquito light traps, 6V battery operated Center for Disease Control (CDC) type, were modified to use carbon dioxide (CO_2) as the attractant. These were tested for a 32-day period from 24 May to 5 July 1974 and subjectively compared to the previous year's methods for evaluating adult population levels.

(b) The results of this study are shown in Appendix F. The investigators felt that indices obtained from the two modified CDC traps with CO_2 as an attractant indicated that the number trapped was more relative to actual population levels and that this technique offered the best way to monitor the effectiveness of control attempts. Observations indicated a CO_2 flow rate of 2 liters per min to be sufficient for trapping adequate numbers of black flies for tabulating indices.

(3) 1975.

(a) An attempt was made during 1975 to refine the CDC modified light trapping technique for adult black fly surveillance. Specifically, a test was designed to determine the flow rate of CO_2 required to effectively attract black flies and to determine the effect of trap location within a small geographic area.

(b) Results are shown in Appendix G. Of the 7 selected flow rates evaluated, the optimum flow rate for trapping black flies in New York was 500 ml of CO_2 per minute. Trap locations in the selected test sites appeared to have little effect on the number of specimens collected. The operating performance of supplying CO_2 from a single source to multiple CDC traps was advantageous.

d. Repellent Jackets.

(1) 1973. Observations on the effectiveness of 35 deet impregnated nylon-cotton one-fourth inch mesh overjackets against black flies were informally conducted during 1973. Field soldiers were asked to wear the jackets and report on their effectiveness, acceptability, comfort, convenience, and irritability. Reports were very encouraging and information gained was used to plan a more comprehensive test for the following year.

(2) 1974.

(a) A determination of the effectiveness and longevity of repellent impregnated jackets as a means of individual protection for the troops at Ft Drum, NY, was made during the period 10 May to 1 July 1974. One hundred and twenty mesh jackets were worn by personnel undergoing combat training field exercises. Based upon questionnaires from soldiers who wore the jackets, an evaluation was made as to effectiveness in preventing black fly bites; skin irritability; and, acceptability with respect to comfort and convenience.

(b) Results of this repellent jacket study are described in Appendix H. While complete protection of persons from black fly bites while training under simulated combat conditions is not possible, the deet impregnated mesh jackets did offer significant protection from black flies as measured by statistical comparisons between the number of bites received by individuals wearing treated and nontreated jackets. The majority of the participants stated that the jacket produced no skin irritability and was comfortable and convenient.

(3) 1975.

(a) Work with the mesh jacket was continued to evaluate experimental US Department of Agriculture (USDA) repellents, impregnated in the cotton-nylon mesh, and to determine their effectiveness in preventing black fly bites. Previous studies had shown that deet impregnated jackets were more effective than topical applications of the same compound. Because deet is becoming increasingly difficult to obtain, its effectiveness is greatly reduced after wetting, and it does not have the longevity of several other repellents, it was considered advisable to investigate the possibility of using other repellents with the mesh jacket concept. Eight experimental repellents were paired with the deet standard and control jackets, and ratios of effectiveness were calculated. Jackets were evaluated in two locations to ascertain the effectiveness against both tropical and temperate species of black flies. During the period 29 January - 11 February 1975, tests were conducted in the central valley of Costa Rica and during 17-24 May 1975 at Ft Drum, NY.

(b) The tests are described and evaluated in Appendix I. Of the 12 experimental repellents evaluated, 2-hydroxyethyl cyclohexane carboxylate (USDA Identification No. AI3-70087) appears to be the best compound for use in impregnating mesh jackets. It was approximately three times better than deet in preventing black fly landings on the upper torso. Two other compounds appeared to be as effective as deet in preventing total black fly landings of tropical species. These were tetrahydrofurfuryl octanoate (AI3-8118) and N, N-Dibutyl-o-ethoxybenzamide (AI3-19084).

e. Laboratory Studies.

(1) A new technical objective was added in 1975 to establish the capability of handling black flies and mermithid parasites in the laboratory as a prelude to biological control studies. The potential of using mermithid parasites has been recognized by many workers. For background information, a survey for natural pathogens and parasites of black flies in the New York study site was made during the period 4-18 May 1975. In collaboration with Dr. William R. Nickle, a nematologist at the USDA Agricultural Research Center, a low level of infection (3-5 percent) was found. Also with the assistance of Dr. George Cantwell, an insect pathologist from the same organization, two species of parasitic microsporidians and one species of fungi infecting the Ft Drum black fly larvae were identified (See Appendix J).

(2) Field experiments with mermithid parasites can be realistically attempted only when procedures have been devised for the mass cultivation of the infective juveniles and when an understanding of mermithid - simuliid interrelationships and effects of modifying environmental parameters is more complete. To mass rear the black fly nematode, a technique must be developed for colonizing large numbers of black flies for *in vivo* development of the parasites, or develop a cell culture for *in vitro* development of the parasite. Studies were directed in both areas. Attempts to get the black fly *Simulium vittatum* to mate and to feed in the laboratory were partially successful using special handling techniques. The newly emerged adults were concentrated in small plastic containers, anesthesized with CO_2 and then placed on a guinea pig. In this manner, about 50 percent would feed and an estimated 5-10 percent mated, but black fly survival for more than 4-5 days after emergence could not be maintained.

(3) A device was fabricated to allow development of large numbers of larvae in a small space. Circular disks of corrugated fiberglass were rotated within a large bell jar by a rheostatically controlled stirring motor (Appendix K).

(4) The most encouraging results in the laboratory has been the development of a self sustaining cell line (Appendix L). Techniques have been developed in the collecting, transporting, storing and processing of eggs to insure aseptic larvae and the production of a continuing primary

cell culture. This opens many possibilities for other workers to study the propagation of pathogens and parasites which might lead to a control of this important insect.

5. CONCLUSION.

a. Three years of field investigative studies on Simuliidae were focused on minimizing the nuisance caused by the biting black flies at Ft Drum, NY. The problem was approached by investigating ground and aerial chemical control measures against black fly adults, and evaluating deet and various experimental repellents, using the mesh jacket concept, as a personal protective measure. Additionally, survey techniques, distribution studies, and binomics were studied to assist in control measures. A survey for natural parasites and pathogens, and laboratory studies of mermithid nematodes were made to examine the possibility of using these agents for biological control of black flies.

b. Little success was obtained using ground chemical control with an experimental rotary tube sprayer. The use of aerial spray was also judged to be infeasible because of the short duration of control produced. Repellent impregnated jackets appear to be the best technique to reduce the degree of nuisance from biting black flies at Ft Drum. Studies showed that deet impregnated jackets were significantly better than topical application of deet and that they had a high degree of troop acceptability. Various experimental repellents were evaluated against both tropical and temperate species of black flies. 2-hydroxyethyl cyclohexane carboxylate (AI3-70087) was judged to be superior to deet and to all other compounds tested. Also the effectiveness of this chemical was not reduced by simulated rainfall.

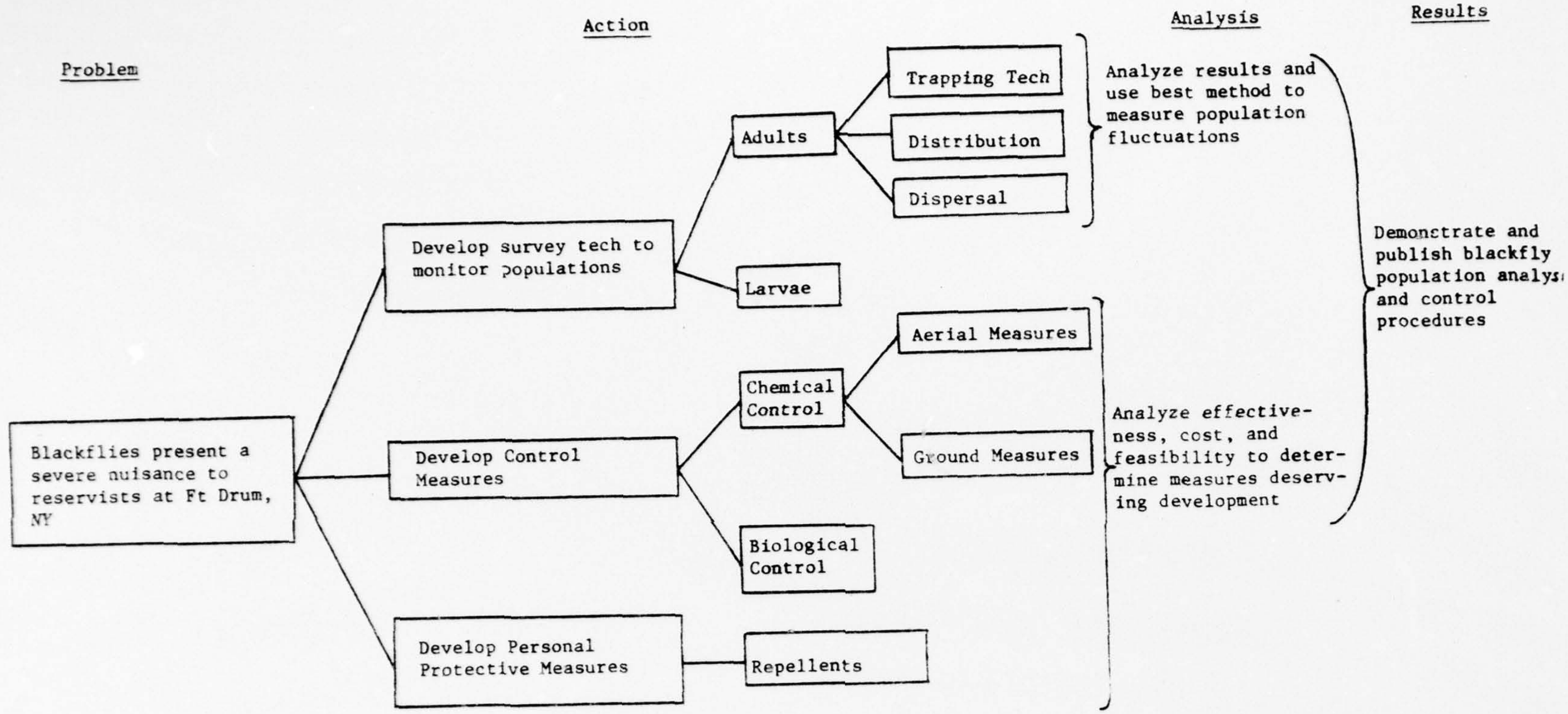
6. RECOMMENDATION. Introduce an insect repellent jacket into the defense supply system as an Army adopted item of medical equipment. Similar repellent jackets, using deet are being produced for commercial use by Cole Outdoor Products of America, 801 P Street, Lincoln, NE 68508.

Bernard A. Schiefer
BERNARD A. SCHIEFER
MAJ, MSC
Chief, Radiation and Entomological
Sciences Branch

APPROVED:

Daniel C. Warren
DANIEL C. WARREN, M. D.
LTC, MC
Chief, Regional Division - North

FIGURE 1. Flow Chart of Blackfly Special Studies Conducted



Rept No. 61-0614-77, Dev of Ground and Aerial Adult Control Measures for Biting Diptera Without Using Persistent Pesticides, March 1973 - June 1976

APPENDIX A

FIELD EVALUATION OF BLACK FLY CONTROL - AERIAL APPLICATIONS - 1973*

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FIELD EVALUATION OF BLACKFLY CONTROL—AERIAL APPLICATIONS [1]

R. R. CARESTIA,[2] R. L. FROMMER,[2] R. W. VAVRA, JR.[2] AND V. A. LOY [3]

To alleviate the irritation and annoyance caused by the blackflies at Camp Drum, New York, a preliminary study was initiated to determine whether relief from annoyance by blackflies could be obtained without using persistent insecticides. The objectives of this evaluation were to determine: (1) effectiveness of aerial dispersal of standard insecticides as adulticides; (2) re-entry time of blackflies after spraying and (3) whether or not aerial spraying could provide 48–72 hours of protection against blackflies.

MATERIALS AND METHODS. The study was conducted for 8 weeks during May and June at Camp Drum, which is a Class I installation of the First US Army; located nine miles east of Watertown, New York. In excess of 90,000 troops, principally from the 15 northeastern states receive their regular 2 weeks annual training here. This training site comprises a total of 107,000 acres and has a maximum elevation of approximately 800 feet. The prevailing winds were southwest to northeast and the mean temperature, relative humidity and windspeed during the evaluation were 61.5° F, 57% and 8.8 mph, respectively. Old New York State Route #26 was selected as the locale for the test site, and a total of 20 square miles was sprayed with different dosages, insecticides and flow rates.

To ascertain what effect aerial spraying of various non-persistent insecticides over 5-square-mile plots would have against blackflies, 4 spray missions were flown by the USAF Special Aerial Spray Flight Unit, Lockbourne AFB, Ohio. Four test plots, plus one control site, each 5-square-miles, were selected for this evaluation. The aircraft used to apply the insecticide solutions was a USAF UC-123K, equipped with a Fairchild Internal Pressure-Spray System (IPSS), with conventional booms and Teejet® flat-fan nozzles ranging from 8003 to 8006 that were set 45° down and into the wind. Line pressures ranged from 40 psi to 85 psi and flow rates were controlled by the size and number of nozzles used. The aircraft operated at a speed of 150 miles per hour, at an altitude of 150 feet for a 500 foot swath interval and at 200 feet for a swath interval of a 1000 feet. The spraying was done in the evenings, commencing about 2 hours before sunset.

The 95% malathion was dispersed through twelve 8003 Teejet nozzles with a boom pressure of 42 pounds psi. Two passes of this treatment were made to disperse a total dosage of 6 oz per acre. The Dibrom® was dispersed through eight 8003 nozzles with a boom pressure of 57 psi. The Dibrom plus Stabilene® combination was dispersed through twelve 8006 nozzles with a boom pressure of 85 psi. The 57% malathion was dispersed through forty-four 8006 nozzles with a boom pressure of 40 psi. The following theoretical dosages were applied on the target areas: 6 fluid ozs. per acre of 95% malathion; 1 fluid oz of Dibrom; 5 ozs. of Dibrom plus Stabilene [4] and 12 ozs. per acre of 57% malathion.

Sampling stations were established and counts of blackflies along with observations of behavior under various parameters of time, temperature, light and wind velocity were accomplished on a daily basis.

Weather conditions such as wind velocity, direction, speed and temperature existing at the training site were determined by the weather station at Camp Drum. In addition, local weather observations at the test sites were also made before, during and after each application.

The spray application was assessed by the exposure of captured flies held in nets (mesh size #30) and by taking in each plot pre- and post- blackfly counts of the natural adult population. To determine the effects of the aerosols on the natural population at least 2 hours prior to each application, the investigators swept three times above their heads with a standard handnet every 15 seconds and took the highest count at 15-minute intervals until the actual treatment occurred. After each sweep, flies were killed by crushing in the nets to prevent recapture. Immediately after the spray, re-entry time was noted by counting the number of minutes elapsing before the flies were seen to return to the investigator. After determining the re-entry time, the investigators then conducted fly counting in the manner described above at 15-minute intervals until dark. This procedure was designated as the "post-count." To determine the kill of trapped flies, adult blackflies were collected in

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[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Dept. of the Army or the DOD. Research sponsored by the US Army Medical Research and Development Command, WASH, DC, 20314, under Contract/Grant No. DA 3A061102B71P01.

[2] U.S. Army, US Army Medical Laboratory, Ft. George G. Meade, MD 20755.

[3] U.S. Army, US Army Medical Laboratory, Ft. Sam Houston, Texas 78234.

[4] Butoxypolypropylene Glycol 100% (8.25 lbs/gal).

standard handsweep-nets and also in the passenger cab area of the investigators' trucks with an aspirator. They were then placed in clean nets and later located on the open road or in the bed of the investigators' trucks. In all tests the blackflies were exposed throughout the spray application.

At least a dozen oil-red dye cards were placed horizontally within each site on metal stands to determine droplet density. Teflon-coated microscope slides were also placed on existent wooden stakes to collect droplets dispersed from the aerial applications. Droplet sizes were computed using the technique described by Yeomans (1949). Sampling stations were spaced at 1 mile intervals along each test site.

RESULTS. Four aerial spray missions were flown during the period 24 May–14 June 1973. On one occasion three applications were applied at three different sites on the same day with the weather and biological parameters being equal.

The effect of aerial spraying as a control measure for adult blackflies was unsatisfactory. The kill of trapped blackflies for all treatments as well as the natural population is seen in Table 1. The data on kill of trapped blackflies along with the natural population at all test sites, as well as weather observations, definitely showed that this poor control was attributed to many variable factors other than re-entry and re-infiltration by blackflies.

At windspeeds above 7 mph it was apparent that the spray applications were not effective and this was directly correlated with reduced fly activity. Temperature was also a factor which significantly influenced blackfly activity as well as light intensity. This was clearly demonstrated by the high rate of kill for trapped flies with the malathion 95% treatment. The temperature at the time of application was 70° F and at the end of the application it was 69° F. All other applications had temperature changes anywhere from 5–13 degrees. It was noted that blackfly activity diminished rapidly with a decrease in light intensity as seen before sunrise and sunset. There was no evidence that humidity was a factor.

The droplet distribution on the dye cards was directly correlated to increased windspeeds during most of the spraying operations. This was evidenced by the fact that some dye cards had adequate coverage whereas others had scattered distribution. There was no difference in droplet distribution for those dye cards placed on the open road and/or under vegetative cover other than that due to increased windspeeds. The droplet sizes for each compound tested are seen in Table 2.

It can clearly be seen that aerial spraying was ineffective, but one must consider the variables associated with blackfly activity, as well as the inherent errors of ULV applications of a pesticide. Thompson (1973) reported that when an effective application rate is found, work should be extended to define that rate in terms of the amount of insecticide in a specific solvent, of droplet size and droplets per given unit area. Perhaps a more dilute solution of the insecticides used would have been more effective for blackfly control. Physical parameters such as temperature, humidity, light and windspeed are very critical to the blackfly and if adult control is to be achieved, the compounds must be applied whenever most of the flies are on the wing. The conditions under which the experiments were conducted were not that ideal.

The adverse weather conditions (wind and temperature) which prevailed at Camp Drum made it difficult, if not impossible, to select an ideal time to treat the sites with the compounds tested. The winds and temperatures at Camp Drum are analogous to the breezes and temperatures one finds at the beach. In the early morning, there is little wind; however, the tempera-

TABLE 1.* Control of natural and trapped adult blackflies, *S. venustum* and *P. hirtipes* with ULV aerial sprays.

Insecticide	Site #	Avg temp	Avg windspeed (mph)	Dose rate (fl oz/acre)	[a] Pre-treatment count per man	[b] Post-treatment count per man	% kill of trapped flies
Malathion 95%	26–3	69° F	8	6	17	18	100
Malathion 57%	26–6	63° F	6	12	5	7	0
Dibrom 14	26–1	60° F	8	1	5	6	30
Dinbrom plus Stabilene	26–R	65° F	9	5	12	14	0
Untreated	26–C	68° F	8	0	16	16	0
Untreated	26–C	63° F	6	0	9	10	0
Untreated	26–C	60° F	8	0	8	8	0
Untreated	26–C	64° F	9	0	15	16	0

[a] Average count per man after taking counts at 15 minute intervals before actual treatment.
[b] Average count per man after taking counts at 15 minute intervals until dark.

TABLE 2. Size of droplets collected with aerial application of insecticides when applied with USAF UC-123K aircraft

Insecticide	Dose (lb per acre)	Volume (fl oz per acre)	Dispersal altitude (feet)	Teejet (no.)	Nozzle (size)	Line (PSI)	Micron diameter range	Average diameter (Micron)
Malathion 95%	0.45	6[1]	150	12	8003	42	5–110	33
Malathion 57%	0.54	12	200	44	8006	40	5–123	35
Dibrom—14	0.10	1	200	8	8003	57	19–114	45
Dibrom plus repellent [2]	0.10/0/25	5	150	12	8006	85	4–237	40

[1] 2 passes at 3 oz per acre.
[2] Stabilene (8.26 lbs/gal).

tures are too low for dispensing insecticides since most of the compounds start to crystallize at temperatures below 70° F. About 9 a.m. the wind picks up and stays rather active with gusts greater than 10 mph until sometime late in the afternoon depending upon whether its direction shifts to direct north or not. In the evening, prior to sunset, the wind dies down, but the temperature also drops making conditions for aerial spraying unsatisfactory. The investigators feel that typical weather conditions at Camp Drum are not ideal for aerial spraying and that new compounds, which are effective at lower temperatures, should be considered for future tests.

It appears that an area greater than 5 square miles is also required to prevent rapid re-entry and infiltration of the natural blackfly population outside of the perimeter not sprayed. Perhaps something like 50–60 square miles is needed to reduce blackfly populations for an area like Camp Drum. Consideration for such control measures may not be economically feasible when one determines the financial support needed.

All of the aforementioned variables concerning ULV dispersal and adult blackfly control need further evaluation. It would appear that observations over several seasons are required to evaluate such factors as windspeed, light intensity and temperature.

Satisfactory control was not achieved with 500 and 1000 foot swath intervals with the Air Force UC-123K aircraft, but additional research needs to be done to determine whether other compounds, with different formulations could be utilized for adult blackfly control programs.

ACKNOWLEDGMENTS. The USAF Special Aerial Flight Spray Unit, Lockbourne, Ohio provided the aircraft, air and ground crews, transport of spray material and certain other facilities used in conducting this evaluation. The authors wish to acknowledge with appreciation the essential part played in the aerial spray missions by LTC Leonard Trager and Major George Rowcliff. Acknowledgment is also made to Dr. Hugo Jamnback, Director, Science Service, University of the State of New York, Albany, New York for his assistance during the initial phases of the experiment. The Medical Research and Development Command, OTSG, Washington, D.C. is also gratefully mentioned for its support in providing the Research and Development funds and equipment necessary for the conduct of the evaluation.

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Rept No. 61-0614-77, Dev of Ground and Aerial Adult Control Measures for Biting Diptera Without Using Persistent Pesticides, March 1973 - June 1976

APPENDIX B

AERIAL CONTROL OF ADULT BLACK FLIES - 1974

Unpublished

Attempts at aerial control of adult black flies were unsuccessful at Camp Drum, NY, in 1973 (Carestia *et al*, 1974). The probable reasons for the failure were improper insecticide aerosol droplet sizes, extreme temperature fluctuation during aerial operations, and use of ineffective insecticide formulations. Since aerial spray operations were not successful, there remained a necessity to develop an effective insecticide formulation for routine and emergency control of black flies.

As with last year's study the US Air Force Reserve 355th Tactical Air Lift Group/Spray Branch, using UC123K aircraft, were the designated applicators. Naled was the insecticide of choice and was applied in two different formulations. Technical grade Dibrom-14® was applied at 1 oz per acre, and a one to nine solution of technical grade Dibrom-14 in heavy aromatic naptha (HAN) was applied at 10 fluid oz per acre; in the latter case the 10 oz per acre dosage rate was obtained by two consecutive applications of 5 oz per acre. With each formulation, 0.103 lb of Dibrom-14 was applied per acre which is in accordance with registered label instructions.

Mean pre- and post-spray black fly population indices were determined using a modified miniature CDC trap with CO_2 as the attractant. Two traps were operated daily from 0900 to 1800 near the center of each test plot. Pre-spray indices were determined for a period of 7 days prior to actual spraying while post-spray indices were recorded daily until the adult populations increased in numbers to a point equal to or greater than the pre-spray index recordings. Immediately prior to the spray missions, three net cages (mesh size #30) containing black flies were placed near the center of each test plot. The purpose of this procedure was to evaluate the kill rate of black flies with respect to each formulation.

Additionally, dye cards and Teflon® coated slides were placed in various locations, perpendicular to the flight path, to determine the distribution and size of droplets being dispersed. Finally, temperature, wind and time were recorded immediately before, during and after each spray mission. The mass median diameter of droplets using Yoemans' (1949) technique was not calculated because it was felt that the confidence interval of the mean was a more statistically valid procedure in this instance.

On 4 June 1974 from 1950 to 2100, the first aerial spray application covering 10,240 acres was conducted. Dibrom-14 was dispersed at a rate of 1 oz per acre through ten 8008 TeeJet flat-fan nozzles set at 45° down with a boom pressure of 47 psi. The aircraft was operated at a speed of 150 mph, at an altitude of 150 feet for a 500 foot swath interval.

®Teflon is a registered trademark of E. I. DuPont De-Nemours and Co, Inc, Wilmington, DE.
®Dibrom-14 is a registered trademark of the Chevron Chemical Co, Richmond, CA.
®TeeJet is a registered trademark of the Spraying Systems Co, Bellwood, IL.

The mean of pre-spray trap indices was recorded at 4,485 specimens. During actual spray operations, the temperature dropped from 75°F at 1950 to 63°F at 2100 with wind conditions remaining near 0 mph. The previously mentioned three test net cages yielded only 60 percent mortality within 20 minutes after exposure to Dibrom-14. This indicates that the application rate used was not entirely effective since netted specimens should have a higher rate of mortality than the naturally occurring population. The rationale for such reasoning is that netted specimens have a greater chance of coming in contact with insecticide droplets either by direct contact, or by indirect contact on the netting. Insecticide droplets from Teflon coated slides were calculated to have a mass median diameter of 49.45μ with a confidence interval of 6.69μ at .05 level of significance. However, since the mean droplet size was considerably greater than recommended 10-25μ (Mount 1970), it is understandable why caged specimens were not effectively killed. Droplets were in all probability not penetrating the net mesh, and only those black flies that came in direct contact with impinged droplets on the netting itself were affected. Dye card results showed the droplets to be evenly distributed, thus suggesting uniform coverage.

On 5 June, the day following spraying, the trap index was 163, clearly showing a significant decrease from pre-spray indices. However, the recordings on 6 June revealed an index of 5235, an increase of 750 specimens over the mean of pre-spray indices. The conclusion based on this increase was that control efforts were only effective for 24-30 hours. This decision was not altered even though the 7 and 8 June trap indices showed a drop to 1600 specimens. It should be noted that daily trap indices vary from day to day and the increase on the 6 June was probably a result of new emergence of black flies.

The second aerial spray mission was conducted on 21 June from 0715 to 0900 over an area of 17,280 acres. The solution of 1 part Dibrom-14 in 9 parts HAN was dispersed through twelve 8010 TeeJet, flat-fan nozzles set at 45° down with a boom pressure of 59 psi at a rate of 5 oz per acre; two passes over the entire area were made resulting in an application rate of 10 oz per acre. The same flight specifications of speed and swath were used as in the first spray application.

The mean of pre-spray trap indices was 1323 specimens. The temperature dropped from 73°F at 0730 to 66°F at 0900 and near zero mph wind conditions prevailed. Unlike the first spray mission, 100 percent mortality was achieved with netted specimens within 20 minutes after completion of spray operations. The initial application of 5 oz per acre of Dibrom-14 in HAN appeared to have no effect on the netted specimens. The reason for such a phenomenon is still unresolved. The mass median diameter droplet size was calculated at 36.71μ with a confidence interval of 8.43 at 0.05 level of significance. The mean droplet size of 36.71μ did not fall within the recommended diameter range; however, a high mortality of caged specimens was achieved. Droplet dispersal was determined by dye card analysis to be even and random, thus, indicating potential uniform aerial coverage.

Effective control was determined to be approximtely 10 days in length. The period of control may have been longer; however, the adult population was already on the decline when aerial spray operations went into effect, and it would be difficult to determine if low trap indices were a result of aerial spray control or natural population decline.

In conclusion, it is apparent that aerial control of adult black flies can be achieved using a Dibrom-14 and HAN mixture as previously specified. The duration of control is somewhat subjective, but a 7- to 10-day control period can be achieved with some certainty.

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APPENDIX C

FIELD EVALUATION OF BLACK FLY CONTROL - GROUND APPLICATIONS*

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FIELD EVALUATION OF BLACKFLY CONTROL – GROUND APPLICATIONS[1]

R. R. Carestia[2], R. L. Frommer[2], R. W. Vavra, Jr.[2], and V. A. Loy[3]

INTRODUCTION. Adequate protective measures against blackflies and other biting diptera at Camp Drum, New York have been needed for many years, but the need now has become more urgent with the reorganization of the U.S. Army and its emphasis on better trained and equipped Reserve and National Guard Forces. The annoyance and irritation caused by blackflies is adverse to morale, training and recruitment, especially during the spring and summer training months.

In May 1973, a preliminary study was initiated to evaluate ultra low volume (ULV) ground applications of nonpersistent insecticides for the control of adult blackflies. In the past five years, numerous field studies have shown excellent results and success with such application for the control of adult mosquitoes as well as other pestiferous insects. Mount et al. (1968) showed that the dispersal of ultra low volumes of undiluted insecticides as nonthermal aerosols using Malathion applied at 0.68 gallon per hour (gph) was as effective against caged adult mosquitoes as diluted thermal aerosols dispersed at 40 gph. Lower costs and less environmental contamination offered by this type of application (Taylor and Schoof 1971) make this technique more desirable and ideal for selection. This reasoning coupled with the recent innovations of commercial ULV dispersal equipment has created considerable interest in this concept for insect control. The following tests were made during the spring and summer of 1973 at Camp Drum, to determine: (1) the efficacy of various aerosols as adulticides; (2) the species composition of blackflies attacking man during the study; (3) the most favorable time of day for ULV spraying; and (4) the re-entry time of blackflies after spraying.

MATERIALS AND METHODS. Seven test sites including one check area were used, each approximately a mile long and 300 feet wide. The following insecticide formulations were applied: (1) 33.8% Malathion plus 14.5% Lethane®; (2) Pyrocide® 7067 (5% Pyrethrum plus 25% Piperonyl Butoxide); (3) Pyrocide X-2749 (5% Pyrethrum, 25% Piperonyl Butoxide plus 5% Repellent 11); (4) 85% Dibrom-14®; (5) 9.3% Dibrom-14 and (6) 6.5% Baygon®. The test area is described as a decidious forest with a combination of dense and semi-dense vegetation consisting mostly of single canopy with some small open areas measuring approximately 100 square yards. A Rotary-Tube Sprayer described and designed by Barnhart and Sheldon (1973) was used for dispersing all of the compounds. The principle of the rotary-tube sprayer is somewhat analogous to the "flit gun", in which air is passed at a high velocity over the tip of a liquid-filled tube. In the rotary-tube sprayer, the tube of liquid is rotated so that its tip moves rapidly through the air. The effect is atomization in both cases. This principle was first developed by Barnhart (1962) at Fort Belvoir, Virginia.

The Rotary-Tube Sprayer has two polypropylene plastic tubes, 1/8 in OD, mounted on a metal bowl set on edge, which is belt-driven. The rotary-tubes are located in the outlet air stream of a 17,000 cu ft/min vanaxial fan, also belt-driven. The air stream serves to move the atomized liquid from the vicinity of the sprayer. A six horsepower gasoline engine powers both fan and rotary-tubes. The speed of rotation of the tubes can be varied between 3,000 to 6,000 rpm. An automotive type tachometer registers the speed.

Liquid to be sprayed is pumped to the rotating bowl by means of an electric fuel pump. The sprayer bowl has a constricted rim. Centrifugal force prevents overflowing and no packing gland or rotary seal is needed where the liquid enters the bowl. The liquid leaves the bowl through two oppositely mounted 1/8 in OD polypropylene tubes, each of such length that the distance from the center of the bowl to the tip of the whip is 11 in. The Rotary-Tube ULV Sprayer was calibrated to deliver three gallons per hour of the material tested with an RPM setting of 5,600.

In order to determine the efficacy of the aerosols tested, prior to each test live flies were collected in hand sweep nets, and also collected in the passenger cab area of the evaluators' truck by the use of an aspirator. Ten flies each were placed in clean, No. 30 mesh nets, positioned behind and/or on top of vegetation approximately three feet above ground at fixed distances downwind from the sprayer. To ensure that the netted flies were not influenced by heat or other adverse factors prior to testing, they were held for at least 30 minutes 200 yards upwind of the sprayer and observed for mortality. Immediately after exposure, the flies were taken out of the bags and placed in clean white enamel pans (12 x 24 x 2) and observed for mortality. During each test, pre-counts of blackflies were made at locations approximately 150 feet into the test site. This was accomplished by sweeping three times above the investigators head every 15 seconds, taking the highest count. After each sweep, flies were killed by crushing in the nets to prevent recapture. After spraying, re-entry time was noted by counting the number of minutes elapsing before the flies were seen to return. After determining re-entry time, a "posttreatment count" was conducted in the manner described above. At least two replicates were used for each compound tested. The passes were made at a rate of three gallons per hour with a vehicle speed of 5 mph. The dosage in lb/acre was calculated on the basis of a swath width of 300 feet (182 acres per hour). This method of recording dosages probably does not give a true measure of insecticidal deposit per acre; however, it does enable investigators to compare one test to another. Droplet size was determined by handwaving

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[2]U. S. Army, U.S. Army Medical Laboratory, Ft. George G. Meade, Maryland 20755.

[3]U. S. Army, U.S. Army Medical Laboratory, Ft. Sam Houston, Texas 78234.

teflon-coated slides at 25 feet from the sprayer according to the method of Yeomans (1949). A minimum of 200 droplets was sampled for each compound tested. Any droplet with a spread factor greater than 1.48 received no correction factor. All tests were conducted in the afternoon during the period 1600-1800 hrs. Temperature and humidity were determined at the test site by thermometers and sling psychrometers. Windspeeds were measured with a Dwyer® wind meter.

To determine what time of day would be most advantageous for spraying, a seven day field monitoring test was conducted at one of the sites. Each day was divided into four time periods as follows: sunrise (0500-0800); noon (1130-1230); afternoon (1600-1630) and sunset (1900-2100). Temperature, humidity, general weather conditions and blackfly activity were noted. Blackfly activity was determined by use of the handsweep-net over and around the investigator's head. During each time period, specimens were collected, mounted and later identified.

Results and Discussion. Results of the ground ULV spraying are shown in Table 1. Although kill was shown for all compounds except Malathion plus Lethane up to distances of 225 feet, the natural populations at all sites were not reduced for any period longer than 3-6 minutes. All compounds were effective but there was a difference in time required to obtain 100% kill. Dibrom-14 in both concentrations was the most effective for quick kill (3 min.), whereas, Malathion/Lethane took the longest time to produce 100% mortality (25 min.). There was no significant difference between the pyrethrum compounds. Table 2 shows the results of the compounds tested against natural population of blackflies. The average temperature was 75°, humidity 65%, windspeed 6 mph.

Table 1.—Kill of netted adult *Simulium venustum* Say obtained with ULV rotary-tube sprayer.

Compound	Discharge gph	No. Tests	Distance (ft) & Kill (%) 50	125	225	300
malathion (38.8%)/ lethane (14.5%)	3	4	100	70	0	0
dibrom 14 (85%)	3	2	100	100	100	0
dibrom 14 (9.3%)	3	3	100	100	100	0
pyrocide 5%	3	3	100	100	70	0
pyrocide x-2749	3	2	100	100	70	0
baygon (6.5%)	3	2	100	100	50	0

Two species of blackflies, *Simulium venustum* Say and *Prosimulium hirtipes* complex were responsible for attacks on man in the test area and were the most persistent and abundant species collected. Figure 1 shows activity at different times of the day. Adult flight activity was influenced by the following: temperature, light intensity, windspeed, rain and other meterological factors. There appeared to be an upper and lower temperature gradient which strongly in-

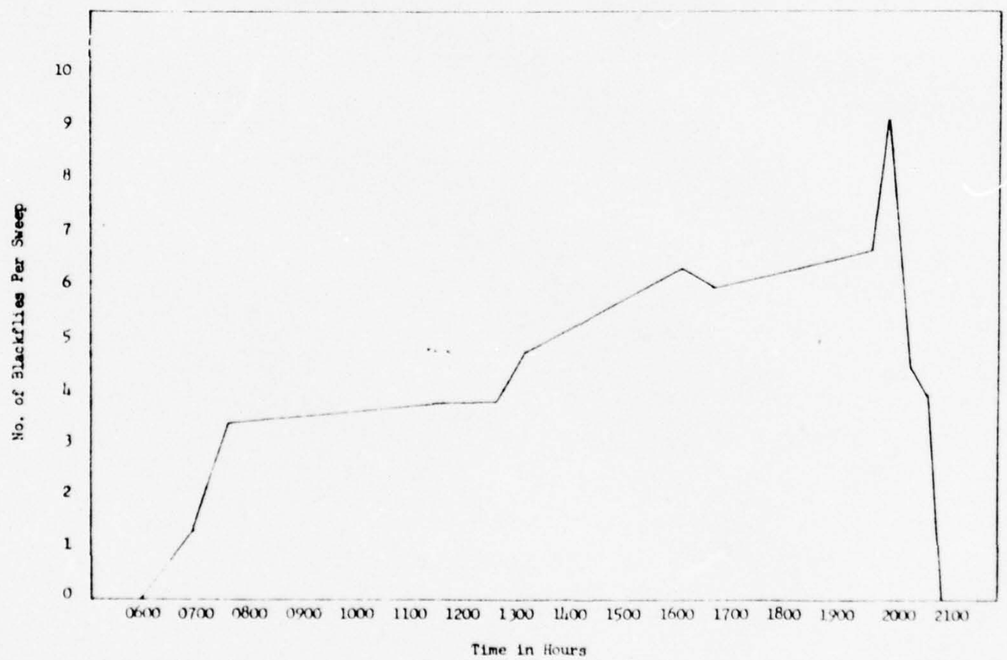

Figure 1. Average daily distribution of blackflies during a seven day period (29 May - 4 June).

Table 2. Data from spraying with ultra low volume aerosols at Camp Drum, N. Y.

Compound	Dosage lb per acre	Re-entry Time Minutes	Avg. Flies per Sweep Pre-spray	Post spray
malathion (38.8%)/ lethane (14.5%)	.062/.018	3	6	7
dibrom 14 (85%)	.230	6	5	7
dibrom 14 (9.3%)	.022	6	7	7
pyrocide (5%)	.007	5	5	6
pyrocide x-2749	.007	6	6	6
baygon (6.5%)	.011	3	5	6

fluenced blackfly activity. Blackflies became active at temperatures above 50°. Windspeeds greater than 7 mph decreased blackfly activity to nil. Observations indicate that only a small percentage of the blackflies in any area are flying at a given time. Most are resting in dense vegetation or other protected sites. When an individual or a vehicle moved into an infested area, at first there was no great sign of blackfly activity; however, within 2-3 minutes the flies appear to sense the host and actively seek him out.

The Rotary-Tube Sprayer used for these tests presented a variable in the test data because of inconsistent performance. The shroud enclosure trapped a considerable amount of material which leaked onto the engine, creating inhalation hazards, and allowed significant loss of material to occur, and the production of larger droplets. Mount (1970) concluded that for effective ground aerosol applications, a mass median diameter droplet size of 5 to 10 μ is optimum to impinge on target insects. Lofgren et al. (1973) conducted tests with soybean oil dispersed by a truck-mounted Leco Model HD® aerosol generator and found that aerosol droplets of 2-16 μ impinged on all body sections of caged mosquitoes, while under field application conditions, droplets of 1-8 μ diameter impinged on outdoor non-caged specimens. In both cases impingement of droplets greater than 16 μ was not obtained. The results of our tests show that most of the aerosol particles produced by the Rotary-Tube Sprayer were not that small and plentiful enough to give adequate coverage, and yet impinged readily on the body surface of the blackflies. Observations on the amount of material trapped in the shroud substantiate these findings even when consideration is given to the inability of the hand-waved slide collection technique to collect droplets smaller than 5 μ (Rathburn 1970). Perhaps the rotary-tube whips should be located outside the shroud to alleviate this problem. The sprayer used failed to give effective penetration into dense vegetation and to adequately clear the vehicle. As a result, there was excessive wetting by spray material on the side of the vehicle after each application. Droplet ranges and mass median diameters of the compounds tested are shown by Table 3.

CONCLUSIONS. Ground ULV applications of nonpersistent insecticides produced kills of trapped adult blackflies at distances up to 225 feet downwind. Dibrom at two concentrations tested was the most effective compound, and required the least amount of time to give 100% kill (three min.). Control of the natural adult population was not achieved: (1) dispersal of the insecticide beyond 225 feet could not be accomplished; (2) re-entry and infiltration from the outer perimeter not sprayed was great; and (3) a significant amount of the material released did not reach the target insect; (4) the particles were too large to penetrate vegetation and to impinge on the blackflies.

Additional research should be done to evaluate the effect of environmental and meterological factors such as temperatures, windspeed, etc. which strongly influence blackfly activity and are directly correlated with the effectiveness of control measures.

Table 3. Mass median diameter and range distribution of ultra low volume ground aerosols tested.

Compound	Range (μ)	Average (μ) Dia.	mmd (μ)	Discharge gph
malathion (38.8%)/ lethane (14.5%)	8-55	19	33	3
dibrom 14 (85%)	10-38	14	24	3
dibrom 14 (9.3%)	19-38	22	28	3
pyrocide (5%)	8-57	18	27	3
pyrocide x-2749	3-76	27	37	3
baygon (6.5%)	8-36	19	28	3

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APPENDIX D

FIELD EVALUATION OF RESMETHRIN AGAINST BLACK FLIES

Unpublished

1. Purpose. To conduct preliminary field evaluation of resmethrin insecticide (effectiveness against adult black flies (Simuliidae) during late June 1973 at Fort Drum, New York.

2. Methods.

a. Adult black flies were collected in the field and placed into small white nylon insect collecting nets for testing against 1.2% aerosolized resmethrin; between 10 and 30 specimens were used for each replicate. During each test, which consisted of three replications, nets were held approximately 2 feet in front of spray can. Resmethrin was then released for a period of 3 seconds, which was estimated as enough time to sufficiently cover and penetrate each net.

b. The effectiveness of resmethrin was determined by observing black fly mortality over given time intervals as presented in Tables 1-3.

3. Results. The results suggested that a 1.2% aerosolized resmethrin will knock down black flies within 1 minute and attain 100% kill against caged adult black flies in approximately 5 minutes under the test conditions reported. However, further field evaluation to include ground and aerial ULV insecticide application, are needed to conclusively determine if resmethrin will effectively and economically control adult black flies in the field.

Table 1. Effect of resmethrin on caged black flies at 2030 hrs 20 Jun 73 at $80^{\circ}F$

 # Flies Dead (morbid) After:

Test Flies		1 min	5 min	15 min
#1	30	5 D/ 25 B*	30 D**	
#2	14	14 B	10 D 4 B	14 D
#3	18	18 B	14 D	18 D

†N - Normal Fly Activity
*B - Down & Buzzing; flies on their backs
**D - Dead (no movement)

Table 2. Effect of resmethrin on caged black flies at 2000 hrs 21 Jun 73 at $70^{\circ}F$

 # Flies Dead (morbid) After:

Test	Flies	1 min	5 min	10 min	15 min
#1	10	10 B*	6 D** 4 B	10 D	
#2	9	9 B	2 D 7 B	9 D	
#3	11	11 B	5 D 4 B	9 D 2 B	11 D

†N - Normal Fly Activity
*B - Down & Buzzing; flies on their backs
**D - Dead (no movement)

Table 3. Effect of resmethrin on caged black flies at 0900 hrs 26 Jun 73 at 75°F

 # Flies Dead (morbid) After:

Test	Flies	1 min	5 min	10 min	15 min
#1	16	13 D** 3 B* 81%	13 D 3 B 81%	14 D 2 B 87%	16 D 100%
#2	20	1 D 19 B 5%	15 D 5 B 75%	16 D 4 B 80%	20 D 100%
#3	16	10 D 6 B 62%	14 D 2 B 87%	16 D 100%	

†N - Normal Fly Activity
*B - Down & Buzzing; flies on their backs
**D - Dead (no movement)

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APPENDIX E

BIONOMICS OF BLACK FLY SPECIES OF FORT DRUM, NY

Unpublished

1. Species Present.

a. *Prosimulium hirtipes* is a holarctic species common throughout New York. It overwinters in the larvae stage. The number of *P. hirtipes* larvae declines rapidly in the spring. They begin biting about a week after emergence and the adults live for about 3 weeks. Members of this complex may be very abundant and are severe bloodsuckers of man.

b. *Prosimulium mixtum*, the first of the pest species to attack man in the spring, is one of three or more very similar appearing species in the "*hirtipes*" group. This species is annoying in the north-eastern United States and eastern Canada. *P. mixtum* breeds in all kinds of unpolluted streams, both temporary and permanent, from tiny trickles to the largest rivers. It is a univoltine species that overwinters primarily in the larval stage; some eggs may hatch late in the winter or even very early spring.

c. *Cnephia mutata* is an early spring species. Larvae of *C. mutata* are found in temporary or permanent streams. It overwinters in the larvae stage and adults of this species are most abundant in May and June. The adults are usually not anthropophilic. It will swarm around and alight on man but rarely feeds on man.

d. *Simulium venustum* is a holarctic species that, in the Americas, is found from Alaska in the west to Greenland in the east and as far south as Texas, Mississippi and South Carolina. *S. venustum* breeds in small, medium and large rivers but is especially abundant in unpolluted turbulent mountain streams, particularly below pools, ponds, lakes and beaver dams. The over-wintering eggs do not hatch until spring when the water temperatures reach 40 - 45° F. This species is multivoltine with generations being annoying well into the summer and a period of newer annoyance in September and October.

2. Parameters Influencing Flight Activity.

a. Temperature. A temperature gradient exists which significantly influences flying activities. Observation during a 7-day continuous period during May - June 1973 demonstrated that the temperature must reach approximately 50° before flight activities begin. Temperatures below this level inhibit flight. In addition, there appears to be a direct relationship between increased temperature and increased flying activities. That is, as the temperature increases, so does the flying population. This continues until the opposite end of the gradient is reached, at which time the flying population diminishes. This appears to occur somewhere above the 85° mark.

b. Light Intensity. The light conditions in a particular area significantly influence the flying population. During the morning period before sunrise (near dark conditions), there is no activity. At sunrise, when there is a dramatic increase in light, flying activities begin, if the temperature is 50° or above. During the evening hours before sunset (near dark conditions) the flies continue their flying activities until sunset occurs. When the light conditions decrease, so does the flying population. This occurs independent of temperature. That is, although the temperature may be within an optimum range for flight, activities rapidly diminish with a decrease in light intensity. After sunset, in dark conditions, no activity occurs.

c. Rain. Any amount of rain appears to significantly reduce flying. During periods of very light rain activity continues to occur at a low level. Any increase above that reduces activity to zero. After periods of continuous rain, i.e., 2 consecutive days, the flying population appears to be heavier than prior to the rain. (Presumably, a large percentage of the population becomes hungry after a lengthy period of inclement weather, and therefore, require a blood meal). Only a small percentage of black flies in an area, are flying at a given time. Most are resting in dense vegetation. This is evidenced by the fact that when moving into an infested area, at first there is no sign of black fly activity. Soon, however, the flies sense the host and actively seek him out from places of resting.

Rept No. 61-0614-77, Dev of Ground and Aerial Adult Control Measures for Biting Diptera Without Using Persistent Pesticides, March 1973 - June 1976

APPENDIX F

A MODIFIED CDC TRAP USING CARBON DIOXIDE FOR TRAPPING BLACK FLIES (SIMULIIDAE: DIPTERA)*

*Published in *Mosquito News*, Vol 34, No. 4, December 1974

A MODIFIED CDC TRAP USING CARBON DIOXIDE FOR TRAPPING BLACKFLIES (SIMULIIDAE: DIPTERA) [1]

R. L. FROMMER,[2] R. R. CARESTIA,[2] R. W. VAVRA, JR.[2]

Snoddy and Hayes (1966), reported the trapping of 11 species of adult blackflies in Alabama over a 6 months period with an average of 34 specimens being trapped per hour, using a modified New Jersey light trap with CO_2 as the attractant. The optimum CO_2 flow rate for attractancy, using a 20 lb (net) cylinder, was 1 pound of CO_2 per hour.

A similar technique in trapping blackflies was conducted at Camp Drum, New York from 24 May to 5 July 1974. However, instead of the New Jersey light trap, a portable 6 volt battery-operated miniature CDC light trap, with light bulb removed, was used. A 20 pound (net) CO_2 cylinder was the source of attractant. A Matheson Gas

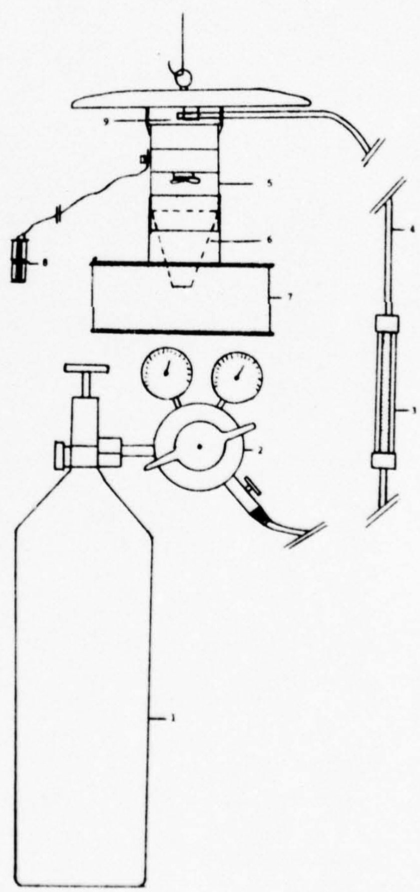

FIG. 1. CDC Miniature Light Trap Modified for Collecting Simuliidae, 1, 20 lb (net Cylinder of CO_2; 2, Regulator Valve; 3, Flowmeter; 4, Tygon Tubing (hose); 5, CDC Trap; 6, Acetate Funnel; 7, Collecting Net; 8, Batteries; 9, Hose Outlet for CO_2.

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Dept. of the Army or the DOD. Research sponsored by the U.S. Army Medical Research and Development Command, Wash., DC 20214, under contract/grant no. DA3A061102B71 Pol.

[2] U. S. Army, US Army Medical Laboratory, Ft George G. Meade, MD 20755.

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Product, 150 mm glass flow meter enclosed in a protective plastic tube and attached at both ends with a ½-inch diameter Tygon tube, 4 ft long regulated the correct amount of CO_2. One end of the tubing was attached to the CO_2 regulator valve outlet while the other end was under the cover plate of the CDC trap (Fig. 1).

A flow rate of 2 liters per minute was found to be the most desirable rate with respect to the frequency of replacing emptied CO_2 cylinders. CO_2 cylinders were replaced on an average of every 5 days. Using Snoddy's technique of one lb CO_2 per hr., replacement would occur approximately every 2 days. The optimum flow rate was not statistically determined. However, the authors feel that the flow of 2 liters of CO_2 per minute is sufficient for trapping adequate numbers of blackflies for tabulating indices.

Each of the 2 traps was constructed with an acetate funnel fused to the plastic sides of the CDC trap. The bottom of the funnel extended about half way down into the cloth trap bag. (Fig. 1. The purpose of the funnel was to prevent loss of specimens due to possible power failure; however, none occurred. The 2 traps were placed 5 ft above ground approximately 150 yards apart in new secondary vegetative growth which is typical of the local fauna and operated for 32 continuous days from 0900 to 1800.

The mean trap index for both traps, over the 32 day test period was 1282. The range of specimens trapped was from a low of 12 to a high of 14,800 with the overall total for both traps being 82,042. See Fig. 2. Identification of species has not been completed.

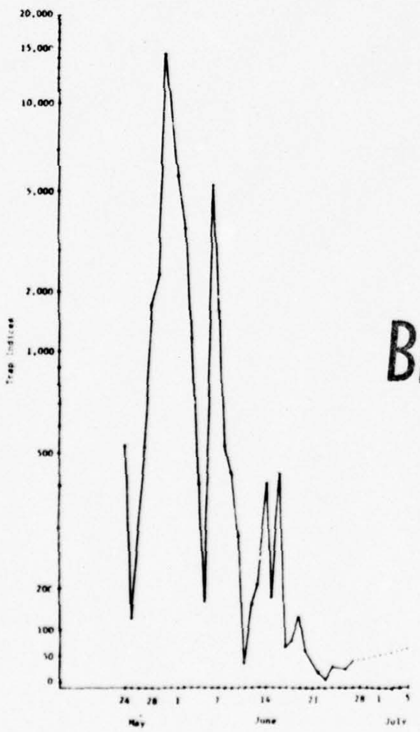

FIG. 2. Adult blackfly trap indices. Indices obtained using 2 modified CDC traps with CO_2 as attractant.

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Rept No. 61-0614-77, Dev of Ground and Aerial Adult Control Measures for Biting Diptera Without Using Persistent Pesticides, March 1973 - June 1976

APPENDIX G

COMPARATIVE EFFECTS OF CO_2 FLOW RATES USING MODIFIED CDC LIGHT TRAPS ON TRAPPING ADULT BLACK FLIES (SIMULIIDAE: DIPTERA)*

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COMPARATIVE EFFECTS OF CO_2 FLOW RATES USING MODIFIED CDC LIGHT TRAPS ON TRAPPING ADULT BLACK FLIES (SIMULIIDAE: DIPTERA)[1]

R. L. FROMMER,[2] B. A. SCHIEFER AND R. W. VAVRA, JR.[3]

Regional Division—North U.S. Army Environmental Hygiene Agency
Fort George G. Meade, Maryland 20755, U.S.A.

ABSTRACT. Seven selected CO_2 flow rates were statistically evaluated using modified CDC traps supplied with CO_2 from a single source. The 500 ml per min (0.1214 lb per hr) flow rate was found to be the optimum for trapping *Cnephia mutata* and *Prosimulium hirtipes*. The operating performance of supplying CO_2 from a single source one 20 lb (net) cylinder via hoses to multiple CDC traps was considered advantageous with respect to ease in selecting specific flow rates, logistics and cost analysis.

Carbon dioxide has been used to collect black flies for many years. Numerous experimenters have evaluated CO_2 as an attractant for black flies using various types of light traps; but, there is little published work analyzing the attractiveness of various CO_2 release rates using valid statistical design. The optimum CO_2 flow rate for attracting black flies has been reported at 1 lb per hr (4.12 liters per min) by Snoddy & Hayes (1966), 2 liters per min (.4857 lb per hr) by Frommer et al. (1974), and 400 ml per min (.0971 lb per hr) by Fallis et al. (1967); however, in these studies, no statistical designs or determinations were reported to validate the findings.

The present study was conducted to analyze statistically selected CO_2 flow rates to determine the optimum CO_2 flow rate for trapping adult black flies. In addition the study was designed to evaluate a method of supplying CO_2 from a single source to multiple operating modified CDC traps.

MATERIALS AND METHODS. The study was conducted daily from 0730 hr to 1400 for 8 days during late May and early June 1975 at Fort Drum, New York. Eight modified CDC traps without light bulbs were fitted with new 2.9v DC motors for use during the study. Four 1.5v flashlight batteries were used per trap as the power source. New motors, which were installed to increase trap suction flow rate performance, were found to have twice the suction (29.2 cfm) compared to the standard CDC Barber-Coleman motors (14.1 cfm). Motors (model F-35c) were purchased from Edmonds Scientific Catalog 752,150 Edscrop Building, Barrington, New Jersey 08007.

All traps used were pretested for equal suction flow rates using an Anemotherm air meter, model 60. The mean flow rate per trap was calculated at 28.2 ± 2.0 ft of air flow per min.

Instead of supplying each trap with a separate CO_2 tank, a single source was used for all eight traps. This consisted of one 20-lb (net) CO_2 cylinder with regulator valve connected by a 3/16-inch bore rubber latex tube to eight interconnected ¼-inch brass T connector valves; eight 50-ft 3/16-inch bore rubber latex tubes led separately from the connector valves to the eight traps (Figure 1). The tubes were clipped under the trap cover plates.

All eight traps were placed 5 ft above

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[1] Research sponsored by the U.S. Army Medical Research and Development Command, Washington, D.C. 20314, under contract no. DA 3A-061102B71P01. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army. Mention of proprietary products is for the purpose of identification only and does not imply endorsement by the Department of the Army.

[2] Present address: 5th Preventive Medicine Unit, APO SF 30301.

[3] Present address: U.S. Army Medical Laboratory, Pacific, APO SF 96343.

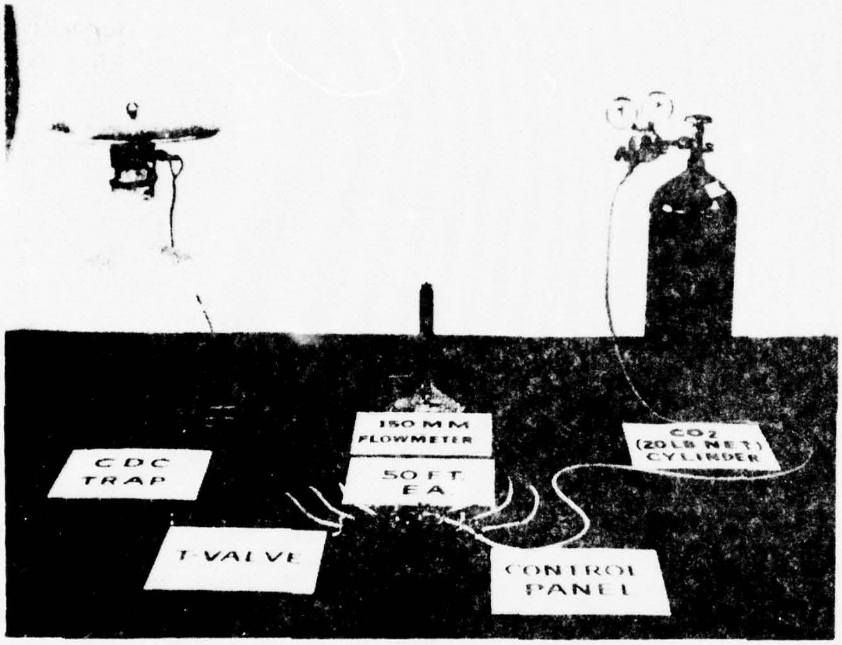

Fig. 1. Apparatus used in evaluating selected CO_2 flow rates in trapping adult black flies.

the ground at random in a 100-ft diameter circle, each approximately 50 ft from the center source of CO_2. The test area consisted of secondary vegetative growth, typical of the local flora.

The following selected CO_2 flow rates were evaluated: 50, 100, 200, 500, 1000, 1500 and 1900 ml of CO_2 per min at 10 psi delivery pressure. A control trap without any CO_2 flow was also operated during each test. Trap flow rates were calibrated at the CO_2 source by making proper valve adjustments using a Matheson Gas Product 150 mm glass flow meter at the beginning and end of each daily test period.

Each CO_2 flow rate was initially assigned at random to the eight traps. Trap flow rates were adjusted daily to ensure that each trap was assigned all seven selected flow rates for a period of 1 day each during the test period. Each trap was also operated for 1 day during the period without CO_2 as a control. This latin square design was used so the variables of trap days, trap locations and selected CO_2 flow rates could be analyzed by use of Duncan's Multiple Range Test. The environmental variables of temperature and wind direction are accounted for by the analysis of trap days and locations respectively. A 10% level of error was chosen in calculating Duncan's Multiple Range Test due to possible fluctuations in each of the CO_2 flow rates.

RESULTS AND DISCUSSION. During testing a total of 4808 adult black flies were collected from all eight traps. The species involved were *Cnephia mutata* comprising approximately 46% of the total and the *Prosimulium hirtipes* complex making the remaining 54%. These species and percentages were determined by the identification of 1234 larval specimens collected in the study site 1 week prior to testing.

An average flow rate increase of 10% was found to occur within each of the seven CO_2 flow rates evaluated. Such fluctuations were anticipated since external temperature can affect both tank and regulator valve setting. The optimum flow rate was difficult to determine since an overlap in the size of trap catches occurred with the various flow rates; however, in this study as little as 500 ml of CO_2 per min (.1214 lb per hr) was found to be sufficient for trapping black flies. A daily mean of 116.88 specimens was collected at this flow rate. Flow rates above 500 ml of CO_2 would waste CO_2 and would not ensure increased trap collecting efficiency. Flow rates below 500 ml of CO_2 occasionally might equal trap results of 500 ml, but on a statistical average would result in fewer specimens trapped (Table 1). The 1 lb of CO_2 per hr that Snoddy and Hayes (1966) reported as the optimum flow rate would be equivalent to 4117 ml of CO_2 per min, which is eight times the optimum delivery rate reported in this study.

The average temperature during the study was 73.2°F ± 3.0°F and the relative humidity was 64% ± 10%. Finally, in monitoring adult black fly activity, trap locations in the selected test site appear to have little effect on the number of specimens collected as shown in Table 1.

Supplying CO_2 from a single source to multiple traps as shown in Figure 1 can be accomplished with considerable ease and accuracy, since all trap flow rates are calibrated at the T-valves and not at the traps. Additionally, this system offers significant savings, since multiple traps can be supplied with varying CO_2 flow rates from a single CO_2 cylinder. The cost of a single 20 lb empty CO_2 cylinder and regulator is approximately $100.00, and the cost of CO_2 is estimated at $6.00 per refill. It was necessary to refill the cylinder every other day since a total flow rate of 5250 ml of CO_2 per min was utilized during testing. A disadvantage of this technique is the requirement of large amounts of tubing if multiple traps are to be operated.

Table 1. Comparison of trap CO_2, flow rates, trap days and trap locations using Duncan's Multiple Range Test.*

Co_2 flow rates in ml/min	0	50	100	200	500	1000	1500	1900
Means**	0.1	24.3	37.8	31.5	116.9	106.8	130.0	153.3
Relationships***	d	cd	bcd	cd	ab	abc	ab	a
Trap days	#1	#2	#3	#4	#5	#6	#7	#8
Means**	203.5	185.0	121.3	33.8	18.0	15.0	12.9	11.5
Relationships***	a	a	ab	b	b	b	b	b
Trap locations	#1	#2	#3	#4	#5	#6	#7	#8
Means**	45.9	76.0	107.0	78.1	65.3	80.3	66.1	81.9
Relationships***	a	a	a	a	a	a	a	a

* 10% level of error.
** Mean number of adult black flies trapped per flow rate, day and location respectively.
*** Means accompanied by the same letter designation are statistically similar.

Days 1 and 2 differ significantly from days 4–8 (Table 1). Light rain occurred throughout the last 4 days of testing, thus reducing adult black fly activity and contributing to this difference. Wind was light and variable (0–10 mph) throughout the study; however, since traps and flow rates were rotated it was assumed that this variable had little influence on the results.

In summary, using the method and design described, adult black flies can be monitored in a small area with logistical ease and accuracy.

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APPENDIX H

FIELD EVALUATION OF DEET-REPELLENT MESH JACKET AGAINST BLACK FLIES (SIMULIIDAE)*

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J. Med. Ent. Vol. 12, no. 5: 558 561 30 December 1975

FIELD EVALUATION OF DEET-TREATED MESH JACKET AGAINST BLACK FLIES (SIMULIIDAE)[1]

By **Robert L. Frommer, Ralph R. Carestia and Robert W. Vavra, Jr.**[2]

Abstract: A cotton-polyester mesh jacket treated with technical N-N-diethylmetatoluamide (deet) was evaluated as a personal protective measure in the prevention of black fly bites. In addition, the jacket was evaluated as to skin irritability, and acceptability with respect to comfort and convenience after continual wear. The deet-impregnated mesh jacket offered significant protection from black flies as measured by statistical comparisons between the number of bites received by individuals wearing treated and non-treated jackets. Of the participants queried, 96.03% stated that the deet-treated jacket produced no skin irritability. With respect to comfort and convenience, a range of 21.9% to 83.2% acceptance was recorded for the jacket.

Seasonally, adult black flies (*Simuliidae*) have interfered tremendously with the training of some 70,000 to 90,000 U. S. Reserve and National Guard personnel during late spring and early summer months at Camp Drum, New York. Black fly attacks accounted for approximately 700 manhr of training time lost in 1 Infantry Division during a 9-day training exercise in 1974.

Previous attempts to control black flies at Camp Drum, N. Y. utilizing several adulticidal formulations proved unsuccessful, due largely to poor weather conditions during aerial operations (Carestia et al. 1974a), and the lack of particle penetration and coverage associated with ground applications (Carestia et al. 1974b). Furthermore under certain environmental conditions, the repellent deet when applied topically to the skin loses some of its effectiveness as a protective measure against biting Diptera (Kahn et al. 1973, Smith et al. 1963).

To provide some relief and protection to training personnel, a 0.634-cm (1/4-in.) mesh cotton-polyester jacket impregnated with deet (FIG. 1) was selected for evaluation. Prior studies demonstrated this preventive concept to offer reliable and effective protection against mosquitoes and other biting Diptera (Catts 1968, Gouck et al. 1967, Gouck & Moussa 1969, Grothaus & Adams 1972, Traub & Elisberg 1962).

This paper describes a field evaluation of the deet-impregnated repellent mesh jacket as a personal protective measure in the prevention of black fly bites.

MATERIALS AND METHODS

This particular jacket was developed by the U. S. Naval Medical Field Research Laboratory, Camp Lejeune, N. C. The jacket is treated with 1/4 g of technical deet (N-N-diethylmetatoluamide) per 1 g net weight of jacket, with the total weight of jacket, without repellent, ranging from 100-110 g. The

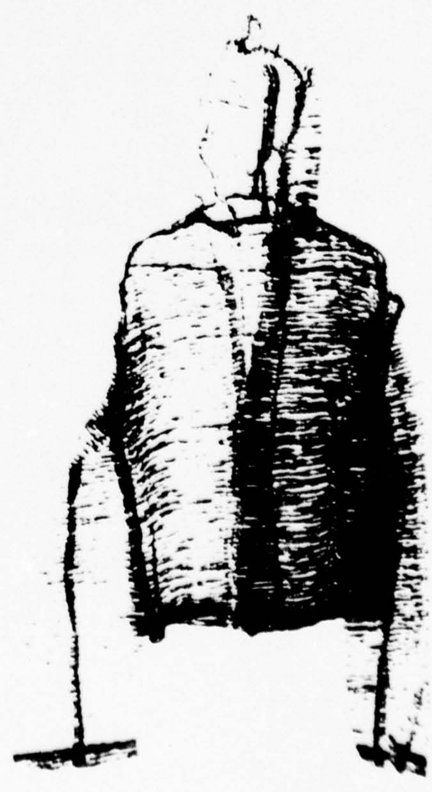

FIG. 1. Deet-treated jacket.

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Dept. of the Army or the DOD. Research sponsored by the U. S. Army Medical Research and Development Command, Washington, D.C. 20314, U.S.A., under Contract/Grant No. DA 3A061102B71P01.

[2] U. S. Army, U. S. Army Medical Laboratory, Fort George G. Meade, Md. 20755, U.S.A.

jacket can be retreated, which under light working conditions is necessary approximately every 6 weeks.

The primary field evaluation of the deet-treated cotton-polyester mesh jacket for protection against adult black flies was conducted from 10 May to 1 July 1974 at Camp Drum, N. Y. This period was one of high adult black fly activity for the region (Carestia et al. 1974b).

The repellent mesh jacket was tested and evaluated under combat training conditions for the following characteristics: (1) its effectiveness as an individual protective measure against black flies, evaluated according to the number of bites received on 6 body areas (head including face, neck, arms, torso above the waist, torso below the waist and legs; (2) acceptability with respect to comfort and convenience after continual wear and (3) irritability to exposed skin.

The 6 body areas where bites were recorded were further segregated into 3 categories to be statistically analyzed. The categories were as follows: (1) the number of bites recorded on all 6 areas of the body; (2) the number of bites on head (including face), neck, arms and torso above the waist; and (3) the number of bites on torso below the waist (including legs).

Sampling with hand sweep nets and trapping using modified CDC traps with CO_2 showed that biting Diptera other than black flies were not present in sufficient numbers to confuse participants in mistaking the source of bites.

During the 6-week test period, 6 different randomly chosen combat units were issued 20 olive drab mesh jackets to be given at random each week to participating soldiers. Ten mesh jackets were deet-treated and 10 were untreated controls. Thus, a total of 120 (60 treated and 60 non-treated) mesh jackets were issued over a 6-week interval. Participants were not told whether they were receiving treated or non-treated mesh jackets. This statistical design helped reduce error resulting from (1) black fly population declines (whether seasonal or from inclement weather conditions) and (2) possible collaboration on part of participants when answering questionnaires.

TABLE 1. Mean number of black fly landing counts recorded on participants testing the following treatments using Duncan's new multiple range test.*

	Treatments			
	1	2	3	4
	Wearing untreated fatigues	Wearing untreated mesh jacket (control)	Wearing deet-treated mesh jacket	Wearing liquid deet repellent on skin and fatigues
Total landings	422	404	6	129
Mean landings**	84 a	80.8 a	1.2 b	25.8 c

*Landing counts were recorded only in the area where the jackets were worn (upper torso) over a single 5-min. interval per day for 5 continuous days.

**Means followed by the same letter are not significantly different at the 5% level of error.

The sampled population was assumed to be normally distributed, as participants were considered to be randomly subject to black fly bites.

New, unused mesh jackets were always issued at the beginning of each week and returned at the end of that week for evaluation. This policy was implemented for the following reasons: (1) to help reduce error from loss of sample size due to lost or misplaced mesh jackets; (2) to reduce error from loss of repellency with time; (3) to reduce error in memory recall in answering questionnaires and (4) to facilitate logistics.

At the end of each week, upon the return of mesh jackets from the field, an evaluation questionnaire (consisting of 31 yes-or-no answer questions) was given to each participating soldier. In each unit participants answered questionnaires collectively while being monitored. This procedure was to help reduce error from possible participant collaboration. Using these data, the acceptability and irritability of the mesh jacket were analyzed statistically using percentages of yes-or-no type answered questions. Differences between the effectiveness of the 2 treatments were analyzed using the paired Student's t-test.

A 10% level of error was chosen because participants had to recall events that occurred during a 7-day period prior to answering questionnaires. Finally, confidence intervals at the 5% error level were fitted to the 2 treatments for comparison of the mean number of bites received per man.

Before conducting the above field study, a 5-day preliminary test was conducted and statistically analyzed by 2 members of the study team to determine the potential effectiveness of the following 4 treatments against black fly bites: (1) deet-treated mesh jacket; (2) non-treated control mesh jacket; (3) standard troop issue deet (75% concentrate) liquid repellent topically applied by hand to the skin (face, neck and hands) and fatigues and (4) untreated skin and fatigues. Black fly landings were recorded on the upper torso (area where the mesh jacket is worn) for a single 5-min. interval per day per treatment. New and unused jackets and fatigues were always worn during each test conducted. Secondarily, the test helped in determining

whether another control treatment would be needed in the primary study.

RESULTS AND DISCUSSION

Since the results of the preliminary field study presented in TABLE 1 revealed that no observable differences existed between the untreated jacket and fatigues, we did not add another control treatment to the primary study. The treated mesh jacket, for which only an average of 1.2 landings was recorded, was considered potentially the most effective protective device against adult black flies, at least under light working or test conditions.

Although the deet liquid repellent when applied topically to fatigues and skin did show some potential protection (25.8 landings), this was considered to be less effective than the treated mesh jacket, as noted from mean comparisons computed from Duncan's new multiple range test.

TABLE 2–4 present the primary results of the effectiveness, acceptability and irritability of both treated and non-treated jackets. The daily time period that each jacket was worn was 4.5 hr, with 98.3% of the participants wearing jackets over their fatigues and the remaining 1.7% over their T-shirts or next to the skin. Taking into account bites recorded on all 6 areas of the body, the treated mesh jacket had significantly fewer bites than the non-treated mesh jacket at the 10% level of significance (TABLE 2). Similar statistical differences were found when exposed areas of the body were tested, such as the torso below the waist and leg regions. However, when comparing treated and non-deet-treated mesh jacket wearers relative to the number of bites acquired in the areas covered by the jacket (head, neck, arms and torso above the waist), the differences between the 2 treatments were not significant. This was probably attributable to participants with the treated mesh jackets not wearing the protective hood, as 56% of all bites with the treated mesh jackets occurred in the head and neck region.

TABLE 2. Statistical analysis using paired Student's t-test on the mean number of black fly bites recorded from participants wearing deet-treated and non-deet-treated mesh jackets in 3 separate categories.*

AREAS OF BODY**	RESULTS OF T-TEST
Covered by mesh jacket	$\frac{4.45-6.31}{1.384} = -1.343$ N.S.
Not covered by mesh jacket	$\frac{0.85-2.06}{0.521} = -2.322$***
All 6 areas of body (total)	$\frac{5.30-8.38}{1.659} = -1.856$***

*Paired Student's t-test in each category consisted of testing the variability existing within and between 60 deet-treated and 60 non-deet-treated mesh repellent jackets over a 6-week period.

**Areas of body are defined as follows: covered by mesh jacket = head (including face), neck, arms, torso above the waist; not covered by mesh jacket = torso below waist and legs; total = all 6 areas of body above and below the waist.

***Differences significant at 10% level of error (2-tailed test).

TABLE 3. Confidence intervals for the mean number of black fly bites per man for individuals wearing deet-treated and non-deet-treated mesh jackets in 3 separate categories.*

TREATMENTS	CONFIDENCE INTERVALS** Deet-treated mesh jacket	Non-deet-treated mesh jacket
Areas covered by jacket (above the waist)	5.30 ± 2.09 bites/man	8.31 ± 2.65 bites/man
Areas not covered by jacket (below the waist)	4.45 ± 1.85 bites/man	6.31 ± 2.09 bites/man
All 6 areas of body (Total)	0.85 ± 0.47 bites/man	2.06 ± 0.86 bites/man

*Each confidence interval tested consisted of examining bites recorded from 60 participants wearing deet-treated and 60 participants wearing non-deet-treated mesh jackets, respectively, over a 6-week period.

**Differences between treatments in all categories significant at 5% level.

Although the t-test may or may not be significant in these comparisons, the use of the confidence interval (C. I.) of the mean offers another perspective of the results in evaluating the overall effectiveness of the mesh jacket under combat conditions. TABLE 3 shows, for all 6 areas of the body, the mean number of bites per man wearing the treated mesh jacket as 5.30 with a C. I. of 3.21 to 7.39 bites, while the non-treated mesh jacket recorded a mean of 8.31 with a C. I. of 5.66 to 10.96 bites per man. This indicates that a person wearing the treated mesh jacket could receive as few as 3.41 bites, while a person with the non-treated mesh jacket could receive up to 10.96 bites under the same test conditions. Again referring to TABLE 3, the confidence interval or the number of bites recorded in the areas covered by the jacket clearly illustrate that a person wearing a treated mesh jacket could receive up to 6 bites fewer than one wearing the non-treated mesh jacket. Finally, in testing the number of bites acquired on exposed areas of the body not covered by the jacket, a participant wearing a treated mesh jacket could have up to 3 bites fewer than one wearing a non-treated mesh jacket. This 3-bite difference indicates that the treated mesh jacket possesses a spacial effect in repelling black flies from non-protected areas of the body.

Although long-term effectiveness was not statistically determined, we feel that under combat conditions, effective protection could be achieved for

TABLE 4. Analysis of the acceptability and irritability of deet-treated and non-deet-treated mesh jacket as determined from questionnaires given to 120 participants.*

	Responses	
	Yes	No
Acceptability		
1. Comfort**	68.3%	31.7%
2. Convenient in walking through brush**	60.7%	39.3%
3. Convenient in crawling through brush**	21.9%	78.1%
4. Convenient in use of weapon**	60.9%	39.1%
5. Interfering while driving tank or Armored Personnel Carrier or jeep**	26.3%	73.7%
6. Interfering while driving 1/2 to 2-1/2 ton truck**	17.9%	82.1%
7. Interfering with helmet**	21.5%	78.5%
8. Interfering with eyeglasses**	16.8%	83.2%
9. Odor offensive (deet-treated mesh jacket)**	21.7%	78.3%
Irritability		
1. Irritable to skin (deet-treated mesh jacket)***	4.0%	96.0%
2. Irritable to skin (non-deet-treated mesh jacket)***	3.8%	97.2%

*Responses are presented as mean percentages of yes-or-no answers.

**Pertains to all participants whether wearing deet-treated or non-deet-treated mesh jackets.

***Pertains to participants wearing either deet-treated or non-deet-treated mesh jackets, as indicated.

up to 3 weeks before retreating the mesh jacket with deet.

Of the 60 participants, 96.0% stated that the deet-treated mesh jacket was not irritable to the skin either as direct skin redness or rash, stickiness from the deet or itchiness of the head (including face, scalp and eyes) and hands. Participants wearing the non-deet-treated mesh jackets had a similarly favorable response of 97.2% (TABLE 4).

Areas of the body where some slight skin irritation occurred (4%) were the head (face and neck) and hands. This was anticipated with the treated mesh jacket since (a) it contains technical deet which might produce some irritation and stickiness and (b) the cotton-polyester mesh material is slightly abrasive. A small percentage (3.8%) of participants wearing non-treated jackets also noted some form of skin irritation. This could have been the result of (a) the cotton-polyester mesh material being slightly abrasive; (b) participant error in answering questionnaires or (c) possible participant exchange of treated and non-treated jackets within each test unit. However, based on personal contact with test unit company commanders, participant exchange of assigned jackets was non-existent.

As shown in TABLE 4, the only training activity for which the jacket was considered unacceptable by participants was crawling through brush (78.1% said inconvenient). This is understandable since the jacket has only 1 securing point, around the hood (FIG. 1). For future wear, excessive wearing, tearing and snagging can be reduced if the jacket is drawn in closer to the body, especially around the waist, chest and wrists. All other areas examined pertaining to comfort and convenience (questions 1–9 in TABLE 4) showed a range of 60.7% to 83.2% acceptance.

Complete protection of persons from black fly bites while training under simulated combat conditions is impossible. Even when wearing the treated mesh jacket, a few bites, 3 to 4, can always be anticipated with some statistical certainty. However, the deet-impregnated cotton-polyester mesh jacket, if worn properly, will give reliable and effective personal protection from biting adult black flies under combat training conditions.

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APPENDIX I

FIELD EVALUATION OF SEVERAL REPELLENTS AGAINST BLACK FLIES (DIPTERA, SIMULIIDAE)*

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FIELD EVALUATION OF SEVERAL REPELLENTS AGAINST BLACK FLIES (DIPTERA, SIMULIIDAE)[1]

B. A. SCHIEFER, R. W. VAVRA, JR., R. L. FROMMER AND E. J. GERBERG[2]

Regional Division—North, U.S. Army Environmental Hygiene Agency,
Fort George G. Meade, Maryland 20755

ABSTRACT. Deet (N, N-Diethyl-meta-toluamide) and twelve promising experimental repellents impregnated in mesh polyester-cotton jackets were tested against black flies in the central valley of Costa Rica and northern New York during 1975. Experimental repellents were paired with a control and a deet standard in each series of tests. Ratios of effectiveness were calculated for relative comparisons. The best compounds tested were 2 hydroxyethyl cyclohexane carboxylate (AI3-70087) and tetrahydrofurfuryl octanoate (AI3-8118).

INTRODUCTION. With recognition of the spatial repellency of impregnated mesh netting, considerable attention has been directed to the use of mesh jackets as a means of providing individual protection from biting Diptera. In the search for effective repellents for impregnation of netting, the majority of research effort has been directed toward mosquitoes both in the laboratory (Gouck et al. 1967b, Schreck et al. 1970) and in the field (Gouck et al. 1967a, Grothaus et al. 1972, 1974). The mesh jacket concept would assume additional importance if adequate protection against black flies (Simuliidae) could be demonstrated. Black flies present a severe nuisance in temperate regions and are involved in the biological transmission of pathogenic organisms to man in tropical areas.

Jackets fabricated of polyester and cotton impregnated with deet were shown to be highly effective compared to topical applications of the same compound against several nearctic species of black flies during the spring of 1974 at Camp Drum, New York (Frommer et al. 1975). It is recognized, however, that deet does not provide all the desired characteristics of the optimum insect repellent. Deet is becoming increasingly difficult to obtain, it does not provide the longevity of many other compounds, and its effectiveness is greatly reduced after wetting (Gouck et al. 1971). It is therefore necessary to screen additional compounds, utilizing the jacket concept, which might prove to be as effective as deet while providing more desirable characteristics of an optimum repellent. The purpose of this paper is to report on two studies designed to evaluate deet and 12 promising experimental repellents against tropical and temperate species of black flies.

METHODS AND MATERIALS. Polyester-cotton, waist length, long sleeved overjackets of .635 cm mesh dyed Army olive drab, shade 107 were used. Each jacket weighed approximately 130 g and was impregnated with 0.25 g of repellent per gram of fabric weight by placing it into a measured solution of the technical grade repellent compound in acetone carrier until the solution was absorbed. After treatment, the jackets were air dried and sealed in plastic bags. Impregnation was accomplished 3 three weeks prior to field testing. The repellent compounds used are shown in Table 1.

Tests were conducted in two locations: in the central valley of Costa Rica during February 1975, and in northern New York during May 1975. In Costa Rica, approximately 30 sites were examined and the 4 sites having the greatest adult black

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[2] Insect Control and Research, Inc., Baltimore, MD, 21228.

Table 1. Repellent compounds and location at which tested.

USDA ENT Code No.	Compound definition	Costa Rica	New York
AI3-2830-Gc	Diisopentyl malate	x	
AI3-3775b	1,3-Propanediol, 2-butyl-2-ethyl 1-ethyl		x
AI3-5974q	1-Butyryl 1-1, 2,3,4-Tetrahydroquinoline		x
AI3-7627Gg	1-Butyl-4-methyl-carbostyril		x
AI3-8118-Gd	Tetrahydrofurfuryl octanoate	x	x
AI3-14825-Gc	1,3-bis (Butoxymethyl)-2-imidazolidinone	x	
AI3-18059-Gd	3-Chloro-1-propanol carbanilate	x	x
AI3-19083-Gb	o-Ethoxy-N, N-dipropylbenzamide	x	
AI3-19084-Gc	N, N-Dibutyl-o-ethoxybenzamide	x	x
AI3-20364-a	1-(o-Ethoxybenzoyl) piperidine	x	x
AI3-20573-Gb	2-[(p-Methoxybenzyl)oxy]-N, N-dipropylacetamide	x	
AI3-22542	N, N-Diethyl-meta-toluamide (deet)	x	x
AI3-70087bGh	2 Hydroxyethyl cyclohexane carboxylate		x

fly activity were selected for the tests. The selected sites were located near Aserri (San Jose Province), Carrizal (Alajuela Province), Navarro (Cartago Province) and Orosi (Cartago Province). The sites at Aserri and Carrizal have been described in greater detail during earlier black fly bionomics studies by Vargas and Travis (1973) as their study sites numbers 4 and 31. In New York, the single study site was located on the military reservation at Fort Drum in Jefferson County near Watertown, New York.

Eight experimental repellents were compared to deet and to an acetone treated jacket (control) in both Costa Rica and New York. The four best repellents from the Costa Rican study plus four additional promising repellents were evaluated in New York. The areas at which the various repellents were tested are shown in Table 1. In each study, local code numbers were assigned to each of the 9 repellent-treated jackets (8 experimental repellents plus deet) and to the control jackets. These numbers were drawn at random to determine the sequence of testing for both participants and repellent jackets.

The procedure for evaluation of the jackets involved four people (2 pairs) seated on stools approximately one meter apart with each participant observing his partner. Two unknown repellents, one standard (deet), and one control were evaluated at one time. Each test consisted of two timed observations of black fly landing counts for the 10 treatments at one location. After each timed observation period, subjects changed locations so the second count was made by a different observer. Ten-minute periods were used in Costa Rica and 5-minute periods were used in New York. After the two observation periods, jackets were changed according to preselected random assignment, and this process continued until all 10 treatments had been tested. Ten new cotton utility shirts, laundered several times, were matched to each repellent mesh jacket to avoid masking of repellents as jackets were changed by participants. The mesh jackets were worn over the shirts and both were stored separately in plastic bags when not in use. Landing counts were recorded in 3 areas of the upper ventral body: (1) jacket only; (2) unprotected face and hands only; and (3) total above waist. These categories allowed statistical comparisons for both spatial and contact repellency. The tests were replicated 10 times in Costa Rica and 12 times in New York.

An additional comparison of the experimental compounds as affected by wetting was conducted in New York. Four replications of observations on the unwashed mesh jackets were made; the jackets were then exposed to the equiva-

lent of 2.54 cm rainfall followed by 4 replications of observations. This treatment was continued for the equivalent of 5.08 cm simulated rainfall. Rainfall was simulated by exposing the jackets in a shower bath. Approximately 30 seconds was the time required to reach an equivalent of 2.54 cm rainfall, approximately 1 m from the shower head. The exact time was determined for each treatment from 3 timed trials by using a standard meteorological rain gauge.

For the purpose of calculating ratios of effectiveness and subsequent statistical analysis of the relative effectiveness of the repellent compounds, a value of one was added to each observation. This was necessary to eliminate occasional negative landing counts on the repellent jacket and to permit the necessary mathematical calculations. Ratios of effectiveness of each repellent to the untreated control and to the deet standard were calculated by dividing the landing count for the two timed observations on the control and deet standard by the paired repellent in each of the 3 areas of observation: landings on jacket only; landings on face and hands only; and total landings above the waist.

An additional transformation of the New York data was made by converting to square roots to compensate for an abnormal distribution. The ratios were reconverted after analysis. The ratios were analyzed using standard analysis of variance of a randomized block design and Duncan's Multiple Range Tests (Duncan 1955).

RESULTS AND DISCUSSION. The tropical species of black flies against which the jackets were tested in Costa Rica included *Simulium metallicum* Bellardi (56%), *S. quadrivittatum* Loew (37%) and *S. callidum* (Dyar and Shannon) (7%). The percentages were determined from collecting samples of landing black flies at each site and are based on a total of 177 identifications. Travis (1974) reported *S. quadrivittatum* to be the predominant species in the central valley of Costa Rica. Both *S. metallicum* and *S. callidum* have been implicated in the transmission of onchocerciasis in Guatemala (Dalmat 1954). The black fly population tested in Costa Rica was much smaller than that observed at Fort Drum, New York. The Costa Rican sites were rather typical of tropical ecosystems where relatively small populations of individual species can occur over long periods of time. The total number of black flies observed landing during the 10 tests in Costa Rica were 2,688. Of these, 2,104 fly landings were made on subjects wearing control jackets.

The predominant temperate zone species of Simuliidae against which the jackets were tested in New York were members of the *Prosimulium hirtipes* complex. Twelve hundred larvae collected 1 week prior to the study from the single study site showed 54% *P. hirtipes* complex and 46% *Cnephia mutata*. However, *C. mutata* rarely attacks man. The total number of black flies observed landing during the 12 tests in New York was 10,756 with 6,399 landing on subjects wearing control jackets.

Black flies are extremely sensitive to environmental changes, and the number of landings varied greatly within a given day and from one day to the next. To minimize the effect of environmental fluctuations, and to provide a more meaningful measure of effectiveness, a random experimental repellent was always paired with the control or the standard repellent. The environmental parameters measured were temperature, relative humidity, and wind. In Costa Rica, the average temperature during the test periods was 22.0°C with a relative humidity of 60.2%. Winds were light and variable from 0–8 km/hr. At the New York test site the average temperature was 26.1°C with a relative humidity of 50% and wind velocity from 0–16 km/hr.

The mean relative effectiveness of the repellent treated jackets to the untreated control and to the standard repellent, deet, is shown in Table 2 for the neotropical species and in Table 3 for the nearctic species.

Table 2. Ranked mean ratio of effectiveness for 20 observations of 10 repellent treatments to control and deet against Costa Rican black flies.

Repellent	Ratio to control			Ratio to deet		
	Total Landings*	Jacket	Face and Hands	Total Landings	Jacket	Face and Hands
8118	7.08 b	5.63 b	3.07 a	1.17 a	0.87 ab	1.40 a
14825	3.51 cd**	3.75 bc	1.90 a	0.65 b	0.69 bc	0.83 bc
18059	5.77 bc	5.67 b	2.68 a	0.37 bc	0.49 d	0.54 cd
19083	3.12 cd	4.19 bc	1.43 a	0.47 bc	0.64 cd	0.57 cd
19084	4.10 bcd	5.49 b	1.42 a	0.79 ab	0.71 bc	0.75 bcd
20364	5.13 bc	5.88 b	2.18 a	0.61 bc	0.78 bc	0.67 cd
20573	2.57 cd	3.39 bc	1.39 a	0.39 bc	0.53 cd	0.51 cd
20830	3.82 bcd	3.50 bc	2.16 a	0.44 bc	0.48 d	0.72 bcd
22542	10.71 a	9.13 a	3.33 a	1.10 a****	1.05 a	1.05 b
control	1.60 d***	1.27 c	1.83 a	0.10 c	0.12 e	0.42 d

* Landings on jacket, face, and hands.
** Means with same letter do not significantly differ (5% level, Duncan's Multiple Range Test).
*** Mean ratio between two control jackets.
**** Mean ratio between two deet jackets.

In the tropical site, deet (AI3-22542) was significantly better than the other 8 repellents tested in preventing black flies from landing on the upper ventral aspect of the body, as well as on the jacket when ranked with the control (Table 2). When ranked with deet, *tetrahydrofurfuryl octanoate* (AI3-8118) and N, N-Dibutyl-o-ethoxybenzamide (AI3-19084) appeared not significantly different from deet in preventing total black fly landings. Also, when ranked with deet, tetrahydrofurfuryl octanoate showed more spatial repellency than deet as indicated by fewer landings on the unprotected hands and face. This compound was also shown to be the most effective chemical against black flies in Maine during the screening of 24 compounds by USDA investigators in 1971 (Gouck, personal communications).

In New York, a previously untested repellent against black flies, 2 hydroxyethyl cyclohexane carboxylate (AI3-70087) was much more effective than other repellents

Table 3. Ranked mean ratio of effectiveness for 24 observations of 10 repellent treatments to control and deet against New York black flies.

Repellent	Ratio to control			Ratio to deet		
	Total Landings*	Jacket	Face and Hands	Total Landings	Jacket	Face and Hands
3775	2.51 cd**	2.74 c	2.36 bc	0.55 cd	0.64 cd	0.57 cd
5974	3.53 c	5.54 b	2.30 bc	1.26 bc	1.95 b	0.99 cd
7627	1.85 cd	1.93 cd	1.32 c	0.38 e	0.46 d	0.40 d
8118	2.62 cd	2.56 c	2.65 bc	0.98 cd	1.04 c	1.13 bc
18059	2.06 cd	2.68 c	1.62 c	0.50 cd	0.74 cd	0.46 cd
19084	1.65 cd	2.07 cd	1.53 c	0.50 cd	0.64 cd	0.52 cd
20364	2.51 cd	2.50 c	1.37 c	0.52 cd	1.02 c	0.48 cd
22542	6.33 b	4.85 b	5.32 b	2.18 b****	1.73 b	1.92 b
70087	18.16 a	10.88 a	13.81 a	5.62 a	4.20 a	3.76 a
control	0.90 d***	0.85 d	0.93 c	0.44 e	0.37 d	0.42 d

* Landings on jacket, face, and hands.
** Means with same letter do not significantly differ (5% level, Duncan's Multiple Range Test).
*** Mean ratio between two control jackets.
**** Mean ratio between two deet jackets.

when ranked with the control (Table 3). Deet was the second best repellent tested in preventing landings on the upper ventral torso. Also, when ranked with the control, 1-Butyryl 1-1, 2, 3, 4-Tetrahydroquinoline (AI3-5974) was as effective as deet in preventing landings on the jacket, but permitted significantly more landings on the face and hands than did deet. When compared to deet, only 2 hydroxyethyl cyclohexane carboxylate was consistently better than deet in preventing landings on the jacket and exposed face and hands.

Rainfall, as expected, had a definite adverse affect on the repellent jackets' effectiveness. Simulated rainfall of 2.54 cm caused an overall 26% reduction in the jackets' effectiveness compared to the control. The effectiveness of deet decreased with wetting more than the other repellents combined. Examination of the landing ratios for each comparison showed 3 compounds whose effectiveness did not appear to be reduced by the total 5.08 cm simulated rainfall. These were repellents 1-Butyl-4-methylcarbostyril (AI3-7627), 3-Chloro-1-propanol carbanilate (AI3-18059), and 2 Hydroxyethyl cyclohexane carboxylate (AI3-70087). Gouck et al. (1971) reported Compound AI3-7627 to remain more than 90% effective after exposure to 6.86 cm rainfall.

This investigation showed that jackets provided better protection in New York than in Costa Rica. Differences in the biting habits of the two groups of black flies attributed to this difference. The Costa Rican species prefer to bite the lower extremities and gradually move to the upper torso only if they are unable to bite in the lower regions. Therefore, additional protection would have to be provided to protect the legs. The small number of flies reaching the face and hands during the Costa Rican test prevented obtaining enough data to clearly show differences in the spatial repellency of those compounds tested. Repellents tested on the much larger black fly population in the New York study area showed significant reductions in black fly landings both on the jacket and on the face and hands.

The repellent 2 Hydroxyethyl cyclohexane carboxylate (AI3-70087) appears to be the best compound for use in impregnating mesh jackets for protection against black fly bites in the temperate zone. Although deet (AI3-22542) and two other compounds adequately protected the upper body against bites from tropical species of black flies, they did not provide adequate protection from black fly bites because of the different biting habits of those flies.

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APPENDIX J

PATHOLOGICAL EXAMINATION OF BLACK FLY LARVAE

Unpublished

1. Aberrant specimens of black fly larvae were collected at Fort Drum, New York State, during the spring of 1975.

2. Abnormal larvae had greatly swollen abdomens and appearing through the integument were large white masses which at first appeared to be fat bodies. Upon microscopic examination of wet mounts, it was determined that the larvae were infected with parasitic microsporidians of two species. One species *Thelohania bracteata* was seen as a group of eight mature sporoblasts in pansporablas and mature spores. The insect infected with this parasite was collected at site #21 on 7 June 1975. Histological preparations stained with hemotoxylin and eosin indicate that practically the entire hemolymph area of the hemoceol was filled with mature spores of this species. Another microsporidian infecting black flies in this area is *Caudospora* sp. The mature spore of this parasite contains a tail-like appendage.

3. Two specimens were collected whose body surfaces were covered with many fine finger-like projections. These individuals were probably dead or moribund when collected. Examination of the slides revealed that the tissues were histolyzed and with very little cellular detail remaining. A fungus, *Saproleguia parasitium*, had penetrated or was growing through the integument.

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APPENDIX K

BLACK FLY REARING METHOD

Unpublished

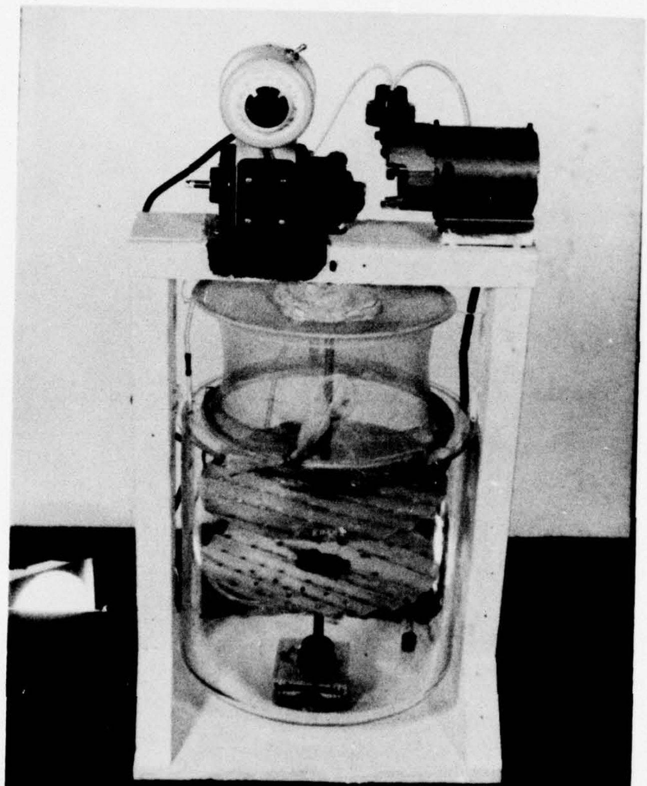
1. A rearing device was fabricated to improve the efficiency in mass rearing *Simulium vittatum*. The rearing chamber consisted of 4 1/2-gallon cylindrical jar (NSN 6640-00-430-1240) with discs, stirring motor, and aerator (Figure).

2. Ten-inch diameter discs were cut from corrugated fiberglass of 1/8-inch thickness. The discs were mounted on a threaded rod and rotated with a variable speed stirrer (T-Line Corp, Model 136-2). Air was supplied through an air stone to the base of the jar with a Model JA 3G001 jewel air pump. A CDC light trap collecting net was secured to the top of the jar to retain the emerging flies.

3. Eggs were placed in the chamber. After hatching, the young larvae spin a silken thread which allow attachment to the discs. The corrugated discs provide a large area for attachment and allows large numbers of black flies to be produced in a small area.

4. Temperature control of the water is desirable. The small size of the apparatus allows its placement within a 12 ft^3 temperature control cabinet. At room temperature, fungus often develops and causes a high mortality of the developing larvae.

FIGURE. BLACK FLY REARING APPARATUS



Rept No. 61-0614-77, Dev of Ground and Aerial Adult Control Measures for Biting Diptera Without Using Persistent Pesticides, March 1973 - June 1976

APPENDIX L

PRIMARY CELL CULTURES DERIVED FROM LARVAE OF *SIMULIUM VITTATUM* (DIPTERA: SIMULIIDAE)

Presented by CPT Jay Abercrombie at the XV International Congress of Entomology, Washington, D. C., 19-27 August 1976 (To be published)

1. Swarms of black flies greatly reduce the working efficiency of people such as soldiers, farmers, lumbermen, and others who work outdoors. When this annoyance is coupled with biting, any outdoor activity is practically impossible.

2. In an investigation by the US Army on possible control methods for black flies, attempts to culture tissues of black flies were made because such cultures should facilitate the study and propagation of black fly pathogens and perhaps lead to their use in controlling this important insect.

3. Eggs were collected in nature in order to initiate the cell line. In the present investigation, *Simulium (Psilozia) vittatum* (Zetterstedt) was chosen for the following reasons: it exists in large numbers in virtually monospecific populations at our study site in Tennessee; large numbers (literally millions) of eggs can be collected in almost any month of the year; and, the eggs remain viable after cold storage and are responsive to laboratory methods to elicit hatching, even after a period of several months.

4. The field study site is Holston Army Ammunition Plant in the Appalachian Mountains of northeastern Tennessee. Coolant water from the explosive manufacturing process is expelled from the plant in a series of fast flowing, high-volume streams. As the streams tumble down slopes to rejoin the Holston River, they provide ideal breeding sites for *Simulium vittatum*. The thermal pollution, with temperatures of 27 to 29° Celsius, and other pollutants in the water result in a depauperate stream fauna; *Simulium vittatum* is apparently one of the few invertebrates which can survive here.

5. Eggs are laid on emergent vegetation or on the leaves that trail in the water from the streambanks. The eggs are rather easily collected in great numbers by gathering the leaves. They were placed on ice and transported back to the laboratory where they were stored at 4° Celsius. The eggs were prepared in order to insure aseptic hatching of larvae by first washing them in a 1:1 mixture of sodium hypochlorite and water for 90 seconds. This freed the eggs from the leaf surface and also from each other and probably also dissolved the gelatin capsule from around the egg. It apparently did not harm the chorion however. If eggs were left in the sodium hypochlorite: water solution for more than 90 seconds they adhered to each other during subsequent steps in the sterilization process, and hence increased the danger of contamination. The eggs were next washed vigorously in a water bath for about 10 minutes on a stainless steel millipore filter screen in order to remove mineral deposits. The eggs were then placed in 70 percent ethanol for 20 minutes and then, under sterile conditions, were washed with distilled water and placed in a small amount of tissue culture medium and maintained at 24° Celsius in an incubator overnight.

6. Hatching usually occurred about 24 hrs later. The young larvae were transferred to a depression slide and each cut into 2 or 3 pieces. From 100 to 200 larvae were thus cut into pieces to initiate a primary culture. The pieces were rinsed 2 or 3 times with culture medium. It is noteworthy that the usual enzymatic pretreatment with trypsin or collagenase apparently is not necessary; in fact, primary cultures initiated without enzymatic pretreatment did just as well as those that underwent this procedure. The pieces were then transferred to 1.25 ml of complete medium, supplemented with fetal bovine serum, in a glass T-9 flask. The medium was slightly modified from that designed by Shields and his co-workers for *Drosophila* and consisted of salts, TC yeastolate in lieu of vitamins, lactalbumin hydrolysate for amino acids, and glucose; sodium bicarbonate was added to adjust the pH to 6.9, the same as their native Tennessee streams. Osmotic pressure was adjusted to 310 miliosmols/liter with sodium chloride. The base for the culture medium was water collected from the black fly's breeding stream in Tennessee and sterilized. The flasks containing the larval fragments were kept at 24^{o} Celsius. The fragments continued to move and contract for about 3 weeks.

7. Cell migration was evident within 10 to 14 days, but was usually confined to areas surrounding the larger larval fragments. The fragments and cells readily attached to the glass substrate but not so firmly as to require trypsin treatment. The medium initially was renewed after about 3 weeks and subsequently at about 2-week intervals depending on the appearance of the culture. Some cell spheres issued from the cut ends of the larval fragments, but they were not as prominent as in *Drosophila* cell cultures as reported by Schneider. Some bleb formation was also noted. Cell growth was not documented until 2 or 3 months of culture and was somewhat slow when compared with primary cell cultures derived from other dipteran species (that is, *Drosophila*, mosquitoes, and house flies). At the present time, after about 10 months, cell replication has increased significantly and production is growing at an increasing rate.

8. Black fly cell in culture show a variety of different forms and types, some of which are quite bizarre. Cells in these cultures were mainly of three different shapes: flat epithelial; ovoid or spherical; and, macrophage-like with membraneous processes. With time, the more diverse cells are apparently eliminated, and ovoid or spherical cells then predominate.

9. Future work is expected to refine the hatching process of aseptic eggs so that larger numbers of larvae may be used to initiate a culture. This should stimulate more rapid growth and permit earlier subculturing. Once this stage has been reached, the possibilities for further studies in cell morphology, developmental biology, genetics, and pathology of *Simulium vittatum* are very promising. The overriding aspiration is to use the cultured cells for propagation of viruses and other pathogens associated with black flies.

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